Archives of Microbiology (2024) 206:7
https://doi.org/10.1007/s00203-023-03731-5

ORIGINAL PAPER

Dynamics of efflux pumps in antimicrobial resistance, persistence, and community living of Vibrionaceae

Sanath Kumar[1] · Manjusha Lekshmi[1] · Jerusha Stephen[1] · Anely Ortiz-Alegria[2] · Matthew Ayitah[2] · Manuel F. Varela[2]

Received: 31 August 2023 / Revised: 30 October 2023 / Accepted: 1 November 2023 / Published online: 29 November 2023

Abstract

The marine bacteria of the Vibrionaceae family are significant from the point of view of their role in the marine geochemical cycle, as well as symbionts and opportunistic pathogens of aquatic animals and humans. The well-known pathogens of this group, *Vibrio cholerae*, *V. parahaemolyticus,* and *V. vulnificus*, are responsible for significant morbidity and mortality associated with a range of infections from gastroenteritis to bacteremia acquired through the consumption of raw or undercooked seafood and exposure to seawater containing these pathogens. Although generally regarded as susceptible to commonly employed antibiotics, the antimicrobial resistance of *Vibrio* spp. has been on the rise in the last two decades, which has raised concern about future infections by these bacteria becoming increasingly challenging to treat. Diverse mechanisms of antimicrobial resistance have been discovered in pathogenic vibrios, the most important being the membrane efflux pumps, which contribute to antimicrobial resistance and their virulence, environmental fitness, and persistence through biofilm formation and quorum sensing. In this review, we discuss the evolution of antimicrobial resistance in pathogenic vibrios and some of the well-characterized efflux pumps' contributions to the physiology of antimicrobial resistance, host and environment survival, and their pathogenicity.

Keywords Efflux pump · *Vibrio* · Virulence · Biofilm · Membrane proteins · Antimicrobial resistance · Cholera

Introduction

The Vibrionales of the phylum proteobacteria are represented by Gram-negative, curved bacteria that inhabit coastal marine environments and are either free-living or live in association with marine crustaceans (Ghenem et al. 2017; Baker-Austin et al. 2018). The cholera epidemic caused by *Vibrio cholerae* is as ancient as the human race. This bacterial microorganism caused seven known epidemics between 1816 and the present, killing millions (Sherman 2007; Deen et al. 2020). As it was known in ancient times, the blue death is associated with the massive cholera-associated diarrhea that turns an infected individual's body into a blue tinge owing to extensive dehydration and consequential capillary rupture (Morris 2007; Wade 2022). The earliest documented cholera-like epidemic could be traced back to Greece in 400 BC (Kaper et al. 1995; Ramamurthy and Ghosh 2021). The true causes of pandemics that ravaged humanity from time immemorial were unknown until Louis Pasteur disproved the theory of abiogenesis in 1862 and unequivocally established the role of microbes in food spoilage and disease, and his counterpart Robert Koch in Germany discovered the causative agents of cholera, anthrax, diphtheria, and tuberculosis (Kaper et al. 1995; Blevins and Bronze 2010).

The causative agent of cholera, *V. cholerae*, is a non-halophilic member of the family Vibrionaceae with over 100 species, 12 of which are associated with human infections more often (Baker-Austin et al. 2018). The *Vibrio* species are oxidase- and catalase-positive, ferment glucose, produce indole from tryptophan, and are motile by polar flagella. The other important species that cause food-borne infections are *V. parahaemolyticus* and *V. vulnificus*. *Vibrio* spp. can cause opportunistic infections such as in wounds, ears, and eyes and septicemia. *V. parahaemolyticus* causes gastrointestinal

Communicated by Yusuf Akhter.

✉ Manuel F. Varela
Manuel.Varela@enmu.edu

[1] QC Laboratory, Post-Harvest Technology, ICAR-Central Institute of Fisheries Education (CIFE), Mumbai 400061, India

[2] Department of Biology, Eastern New Mexico University, Station 33, Portales, NM 88130, USA

infections associated with consuming raw or undercooked fish and shellfish. In contrast, *V. vulnificus* causes wound infections and septicemia. These *Vibrio* spp. are widely distributed in coastal-marine water, sediment, plankton, and aquatic animals (Colwell et al. 1977). Other *Vibrio* spp. that are involved in sporadic food-borne intestinal or extraintestinal infections include *V. furnissi*, *V. mimicus*, *V. anguillarum*, *V. fluvialis, V. damsele, V. furnissi,* and *V. metschnikovii* (Colwell et al. 1977; Morris 2003; Baker-Austin et al. 2018). *V. fischeri* is a well-known endosymbiont of light organs of squids, and *V. harveyi* is a pathogen of marine crustaceans, particularly shrimp. Other *Vibrio* species are also opportunistic pathogens of different life stages of fish and shellfish.

The vibrios, except *V. cholerae* and *V. mimicus*, are halophilic. Their numbers increase with increasing temperature in temperate waters, with the high numbers recorded during warm summer months when the temperature is > 25 °C, which also correlates with the higher rates of infections (Motes et al. 1998; Hounmanou et al. 2023). Infections are often associated with the consumption of raw seafood containing high (> 10^5 CFU/g) numbers of vibrios, particularly the filter-feeding bivalve mollusks, which accumulate bacteria to concentrations several folds higher than that in the surrounding water (Baker-Austin et al. 2018). Vibrios respond uniquely to unfavorable environmental conditions such as very low temperatures and high salinity by entering into a dormant state called "viable but non-culturable" (VBNC) state (Colwell 2000; Oliver 2010). In the VBNC state, bacteria are metabolically inactive. These bacteria do not grow on standard laboratory media but retain their ability to infect and cause disease (Huq and Colwell 1996).

Epidemiology of *Vibrio* spp.

Vibrio cholerae

V. cholerae remains the most devastating *Vibrio* spp., still responsible for water-borne infections and deaths in developing countries. About 3–5 million cholera cases with 100,000 deaths are reported annually (Deen et al. 2020; Saha and Ganguly 2021; CDC 2023). Cholera is endemic in 51 countries, particularly in Sub-Saharan Africa and Southeast Asia (Harris et al. 2012; Ali et al. 2015; Guillaume et al. 2018). Although over 200 serotypes of *V. cholerae* have been identified based on the lipopolysaccharide antigens (Morris 2003), the severe form of cholera known as "*cholera gravis*" is caused by *V. cholerae* O1, identified by its ability to agglutinate with the O1 antiserum. All other *V. cholerae* that do not agglutinate with O1 antiserum are termed "non-O1." The pandemic strains of O1 are further grouped into "Classical" and "El Tor" biotypes (Kaper et al. 1995; Reidl and Klose 2002). Epidemiological studies suggest a succession of these biotypes in cholera pandemics. The Classical biotype caused the first six pandemics, while the ongoing pandemic involves the El Tor biotype (Mukhopadhyay et al. 2014). Based on the antigenic differences, these are further subdivided into Ogawa, Inaba, and Hikojima serotypes (Kaper et al. 1995). The severity of cholera caused by *V. cholerae* O1 is due to cholera toxin production (Kaper et al. 1995). The non-O1 strains do not produce cholera toxin; hence, they are non-pathogenic or cause mild gastrointestinal infections. However, certain strains are occasionally involved in severe cases of intestinal and extraintestinal infections (Bag et al. 2008; Mukherjee et al. 2014).

In 1992, a non-O1 serotype involved in a cholera outbreak similar to that caused by O1 *V. cholerae* was attributed to the serotype O139, which also produced the cholera toxin (Ramamurthy et al. 1993). This strain is the only non-O1 *V. cholerae* reported thus far to be capable of causing a severe form of cholera as the O1 serotype. Although *V. cholerae* O139 reportedly originated from the El Tor strain, they are quite distinct antigenically (Albert et al. 1993; Faruque et al. 2003b). Large cholera outbreaks occurred in India and Bangladesh due to the O139 serotype in 1992 (Albert et al. 1993; Nair et al. 1994). However, subsequently, this serotype has not been responsible for any new cholera cases, and the El Tor biotype has caused almost all cholera infections worldwide. Recently, Yemen has been the focal point of large outbreaks involving over 364,000 cases and 639 deaths in 2019 (Deen et al. 2020; UNHCR), while countries such as Afghanistan, DR Congo (Ingelbeen et al. 2019), Haiti (Rubin et al. 2022), Ethiopia, and Cameroon continue to report the most cases (Deen et al. 2020; WHO 2022; ECDC 2023).

The virulence of *V. cholerae*, by far, relies on its ability to elaborate cholera toxin, CT, an A-B type toxin with one A subunit and five B subunits, which exerts its lethal effects on the physiology of intestinal epithelial cells by NAD-ribosylation of the adenylate cyclase complex leading to uncontrolled production of cyclic AMP (cAMP) molecules (Kaper et al. 1995). This results in continuous loss of water and electrolytes from the cells, which manifests as the classical rice water stool of cholera. In addition to CT, the Zonula occludens toxin (Zot), the accessory cholera enterotoxin (Ace), and a core encoded pilin (Cep) are important for the virulence of *V. cholerae*. Other accessory virulence factors that contribute to the ability of *V. cholerae* successfully to colonize the intestinal epithelium include the toxin-coregulated pilus (TCP), regulatory proteins like ToxR, membrane porins, iron-regulated outer membrane proteins, the O-antigen of the lipopolysaccharide, and accessory colonization factors (Faruque et al. 2003a).

The non-O1, non-O139 *V. cholerae* (NOVC) serotypes are involved in intestinal and extraintestinal infections (Dutta

et al. 2013; Deshayes et al. 2015; Vezzulli et al. 2020). Although NOVC lacks CT and TCP, they possess other putative virulence factors such as a heat-stable toxin (NAG-ST), a hemolysin (Hly), RTX toxins (Repeats in ToXin), hemagglutinin protease (HapA), toxin regulatory protein (ToxR), outer membrane proteins (Omp), and the type III (T3SS) and type VI (T6SS) secretion systems. However, these factors are not consistently present in all NOVC isolates (Bag et al. 2008; Ceccarelli et al. 2015; Neogi et al. 2019). Reported cases of NOVC bacteremia involve persons with predisposing conditions such as liver cirrhosis, diabetes, and malignancy, with seafood and water as major sources of infection (Chen et al. 2015; Deshayes et al. 2015). Most reported cases of NOVC involved acute gastroenteritis, biliary tract infection, and primary bacteremia. In contrast, occasionally, peritonitis, skin and soft tissue infection, and urinary tract infection have been reported (Chen et al. 2015).

In 1998, investigation of an unusual surge in the incidence of cholera-like infections involving non-O1 *V. cholerae* in Calcutta, India, revealed the involvement of serotypes such as O144, O11, O6, O8, O12, O19, O39, and O58 that exhibited distinct cytotoxic effect on CHO and HeLa cells, but uniformly lacked any virulence factor associated with O1/O139 *V. cholerae* (Sharma et al. 1998). Case studies have reported the involvement of non-O1 *V. cholerae* in pulmonary infection (Shannon and Kimbrough 2006), pneumonia (Marinello et al. 2017), septicemia with meningitis (Hao et al. 2015), necrotizing fasciitis (Ottaviani et al. 2011), and traumatic wound infection (Hirota et al. 2010).

Vibrio parahaemolyticus

V. parahaemolyticus is a Gram-negative, halophilic bacterium widespread in coastal waters and seafood. Fujino first discovered the microorganism following an outbreak of food poisoning in 1950 associated with consuming partially cooked sardine (*shirasu*) in Japan (Chakraborty et al. 1997). These microorganisms are natural inhabitants of inshore marine waters of temperatures above 15 °C, varying salinity and estuaries, and prevalent in warm summer (DePaola et al. 2003; Ndraha and Hsiao 2019; Fernández-Vélez et al. 2023).

V. parahaemolyticus is a major cause of seafood-borne illness worldwide, characterized by gastroenteritis and wound infections. The disease is distinguished by diarrhea, headache, vomiting, nausea, abdominal cramps, and low fever. Consumption of raw or partially cooked seafood, especially bivalves like oysters and clams, is frequently associated with food-borne infections caused by *V. parahaemolyticus*. The pathogenic strains of *V. parahaemolyticus* are distinguished by the presence of hemolysins, thermostable direct hemolysin (TDH) factor, and/ a TDH-related hemolysin (TRH) encoded by *tdh* and *trh* genes with about 70% nucleotide sequence similarity (Nishibuchi et al. 1985, 1989). A pandemic clone of O3:K6 harboring the *tdh* gene but not *trh* has been responsible for many outbreaks in Asia and the US since its first report from Kolkata, India, in the mid-nineties (Okuda et al. 1997).

Subsequently, other serotypes such as O1:KUT, O4:K68, and O1:K25 were also found to belong to the pandemic group of *V. parahaemolyticus*, presumably evolved from O3:K6 serotype since all these serovars were genetically indistinguishable based on their arbitrarily primed PCR, ribotyping and pulsed-field gel electrophoresis (PFGE) patterns (Okuda et al. 1997; Matsumoto et al. 2000; Okura et al. 2003; Chowdhury et al. 2004). The pandemic clones are characterized by specialized genetic traits that distinguish them from pre-pandemic strains of the same serovars, such as the presence of the *tdh* and the absence of the *trh* gene and a characteristic *toxRS* sequence (*toxRS/new*) with point mutations that can be used to detect the pandemic clones using a group-specific PCR (GS-PCR) (Matsumoto et al. 2000). Recently, a new type of *V. parahaemolyticus,* "O4:KUT-recAin," with a new type of K antigen and a 25 kb genomic island inserted in the housekeeping gene *recA* has been reportedly a predominant cause of infections in China (Chen et al. 2020).

The *tdh*- and *trh*-positive *V. parahaemolyticus* are frequently encountered in marine fish and shellfish. Bivalve mollusks such as clams, oysters, scallops, etc., are important sources of human infections since these filter-feeding organisms accumulate *V. parahaemolyticus* in their muscle to levels much higher than in the surrounding water and sufficient to cause human infections (Deepanjali et al. 2005; HongYou et al. 2014; Mok et al. 2019; Hong To et al. 2020). Wild-caught and farmed shrimp are also important vehicles of pathogenic *V. parahaemolyticus* (Ayyappan et al. 2018; Changsen et al. 2023). *V. parahaemolyticus* has been recognized as the causative agent of acute hepatopancreatic necrosis disease in shrimp, further emphasizing this bacterium's increasing importance as a zoonotic pathogen (Hong To et al. 2020).

Vibrio vulnificus

V. vulnificus can cause a range of infections, including gastroenteritis, sepsis, necrotizing fasciitis, and cellulitis, with a case fatality rate as high as 50%, particularly when susceptible individuals with underlying debilitations such as chronic liver disease and iron overloaded-condition (Kathleen et al. 2016; Heng et al. 2017). *V. vulnificus* is responsible for over 95% of the fatal cases of seafood-borne infections in the US (Kathleen et al. 2016). Nearly 25% of *V. vulnificus* infections are associated with the exposure of open wounds to seawater containing this pathogen (Bross et al. 2007).

Like all other *Vibrio* spp., *V. vulnificus* is widely distributed in coastal-marine water associated with the chitinous

exoskeleton of marine crustaceans and gets concentrated to infectious levels in filter-feeding bivalve mollusks (Heng et al. 2017; Bonnin-Jusserand et al. 2019). While infections occur with raw or undercooked seafood consumption, wound infections leading to bacteremia can occur when exposed to seawater containing these bacteria or while handling seafood. The abundance of *V. vulnificus* increases with seawater warmer above 20 °C and at a moderate salinity of 15–25 ppt in temperate waters (Motes et al. 1998).

In contrast, in the tropics, *V. vulnificus* abundance is influenced more by salinity than the water temperature (Parvathi et al. 2004). At lower temperatures (< 13 °C), the bacterium enters into a viable but non-culturable (VBNC) state (Oliver 2010). Of three known biotypes of *V. vulnificus*, biotype 1 is predominantly responsible for human infections, biotype 2 is an eel pathogen, and biotype 3, a hybrid between biotypes 1 and 2 first reported in Israel, is a pathogen of freshwater fish such as tilapia and carps. However, the microorganism has been reported to cause human infections, such as severe soft tissue infections, with a relatively lower mortality rate than biotype 1 (Zaidenstein et al. 2008; Jones and Oliver 2009; Oliver et al. 2012). Climate change leading to an increase in seawater surface temperature is predicted to dramatically increase the cases of *V. vulnificus* infections in the future (Archer et al. 2023).

Unlike *V. cholerae* and *V. parahaemolyticus*, no specific virulence factor has been associated with the pathogenesis of *V. vulnificus*. Some of the important virulence factors include a cytotoxic pore-forming toxin (VvhA; *V. vulnificus* hemolysin), capsular polysaccharide (CPS), and a multifunctional autoprocessing repeats-in-toxin (MARTX) (Jones and Oliver 2009; Yuan et al. 2020; Li and Wang 2020). Other putative virulence factors include metalloproteases, collagenases and phospholipases, siderophores, fimbriae, and others (Gulig et al. 2005; Jones and Oliver 2009; Horseman and Surani 2011).

Antimicrobial resistance in *Vibrio* spp.

Antimicrobial agents are indispensable in human clinical medicine and have saved humankind from life-threatening microbial infections and continue to do so. Apart from being a therapeutic drug, these agents are also administered in subtherapeutic doses among livestock, poultry, and aquaculture to prevent disease and promote growth (Lekshmi et al. 2017). Consequentially, imprudent use of these antimicrobial agents leads to the development and spread of bacterial antimicrobial resistance (Loo et al. 2020). While the use of antibiotics in aquaculture for the treatment of bacterial infections or as a prophylactic measure can promote the development of antibiotic resistance in *Vibrio* spp., the antibiotic residues in farm effluents can have a similar effect on the bacteria in the environment surrounding aquaculture farms. Although vibrios are usually sensitive to antimicrobials of veterinary and human importance (Kumar et al. 2017), there are incidence reports of multidrug-resistant vibrios with different antibiotic susceptibility profiles in clinical settings (Reyhanath and Ranjeet 2014; Tan et al. 2020). The antimicrobial resistance in vibrios in wild-caught and farmed fish and shellfish is a serious threat due to their zoonotic potential and the dissemination of such bacteria in the consumer community through the food chain (Martínez 2008).

The massive diarrheal condition observed in cholera patients causes dehydration and could be fatal if not treated early. Effective management of cholera involves oral rehydration, and antibiotic treatment is generally not warranted. However, antibiotics can drastically reduce the fecal shedding of *V. cholerae* and help prevent environmental contamination and transmission of cholera. *V. cholerae* is generally sensitive to frequently prescribed antimicrobials, including β-lactams, aminoglycosides, tetracycline, and quinolone antibiotics.

However, the incidences of antimicrobial resistance in cholera and non-cholera vibrios have increased (Costa et al. 2021). This condition can be attributed to their antibiotic exposure and the acquisition of antibiotic-resistance genes from other Gram-negative bacteria in the environment. Tetracycline and azithromycin have been the drugs of choice for treating cholera for close to four decades until the first emergence of tetracycline resistance in Bangladesh in 1979, in which the O1 isolates were also resistant to ampicillin, kanamycin, streptomycin, and trimethoprim-sulfamethoxazole (Glass et al. 1980).

On the other hand, several prior studies reported the isolation of antibiotic-resistant *V. cholerae*, such as biotype El Tor, from cholera cases (Kuwahara et al. 1967; Prescott et al. 1968). Rahal et al. (1973) reported *V. cholerae* resistant to tetracycline, chloramphenicol, sulphonamides, ampicillin, streptomycin, and kanamycin in Algeria (Rahal et al. 1973). Nearly 76% of *V. cholerae* El Tor strains isolated during the fourth epidemic outbreak in Tanzania were resistant to tetracycline, a phenomenon attributed to the extensive therapeutic and prophylactic use of tetracycline. (Towner et al. 1980). Following this, resistance to tetracycline and other commonly employed antibiotics in cholera therapy was reported from different geographical regions worldwide (Threlfall et al. 1980; Ichinose et al. 1986; Ouellette et al. 1988; Tabtieng et al. 1989; Saraswathi and Deodhar 1990; Dalsgaard et al. 2000).

V. cholerae O1 El Tor serotype Ogawa isolated in Honduras were resistant to tetracycline, trimethoprim-sulfamethoxazole, kanamycin, gentamicin, chloramphenicol, ampicillin, cephalothin, and doxycycline (Dubon et al. 1997). Over 75% of the *V. cholerae* O1 strains isolated in Kolkata, India, were resistant to tetracycline (Bhattacharya et al. 2011).

Multidrug-resistant *V. cholerae* El Tor Biotype, Ogawa serotype resistant to tetracycline, trimethoprim, co-trimoxazole, nalidixic acid, polymyxin B, spectinomycin, streptomycin, and sulfamethoxazole was reported from a cholera outbreak from Odisha, Eastern India in 2010 (Jain et al. 2016). Similarly, *V. cholerae* O1 El Tor Ogawa isolated from 2012 to 2015 cholera outbreaks in Mozambique were 100% resistant to sulphamethoxazole-trimethoprim, while more than 90% of the isolates were resistant to ampicillin, nalidixic acid, chloramphenicol, and nitrofurantoin, and were susceptible to azithromycin and streptomycin (Dengo-Baloi et al. 2017). In recent times, frequent isolation of *V. cholerae* O1 and O139 strains resistant to all clinically employed drugs has been reported from different parts of the world (Verma et al. 2019; Chatterjee et al. 2020).

Antimicrobial resistance is being reported regularly among vibrios of pathogenic importance. The β-lactamase-associated resistance mechanisms were identified in *V. harveyi*, *V. alginolyticus,* and *V. parahaemolyticus* associated with seafood in Italy (Ottaviani et al. 2001). In Mexico, 70% of *Vibrio* isolates from penaeid shrimp were resistant to carbenicillin and ampicillin and harbored resistance plasmids (Molina-Aja et al. 2002). Multiple antimicrobial resistance was also reported in *Vibrio* strains from wastewater final effluents, indicating the spread of resistance genes in a sewage plant, posing a threat to communities dependent on such water bodies (Okoh and Igbinosa 2010).

An investigation on farmed fishes from South Korea during 2005–2007 reported drug resistance in *V. parahaemolyticus* and *V. alginolyticus* with maximum resistance against ampicillin (Oh et al. 2011). Multi-drug resistance was also observed in both strains, with a higher incidence in *V. alginolyticus* (Oh et al. 2011). Studies from across the globe have reported plasmid-mediated resistance against clinically relevant antibiotics in vibrios from wild-caught and farmed fish and shellfish (Manjusha and Sarita 2011; Reyhanath and Ranjeet 2014; Letchumanan et al. 2015; Loo et al. 2020). Antibiotic resistance with an average MAR index of 0.77 among the *Vibrio* isolates from crustaceans of retail markets has been reported from Egypt (Ahmed et al. 2018).

Several reports suggest that *V. parahaemolyticus* is becoming increasingly resistant to previously effective antibiotics such as tetracycline, aminoglycosides, cephalosporins, fluoroquinolones, and others (Elmahdi et al. 2016; Dutta et al. 2021; Grudlewska-Buda et al. 2023). Irrespective of the geographic region, resistance to ampicillin, penicillins, and tetracyclines is observed in *V. parahaemolyticus* worldwide (Elmahdi et al. 2016). *V. parahaemolyticus* isolated from seafood in Malaysia exhibits resistance to ampicillin, cefazolin, and penicillin. Resistance to more than one antibiotic was found in 90% of the isolates, with the MAR index ranging from 0.04 to 0.71 (Tan et al. 2020). As high as 24% of the seafood samples in Bulgaria were found contaminated with *V. parahaemolyticus* resistant to ampicillin, cefepime, and ceftazidime, with the MAR index ranging from 0.10 to 0.30. (Stratev et al. 2023). A study on the market samples of seafood sold in Vietnam reported 86% of *V. parahaemolyticus* isolates to be resistant to at least one antibiotic tested, with the highest resistance observed against ampicillin, followed by cefotaxime, ceftazidime, trimethoprim-sulfamethoxazole, and tetracycline (Vu et al. 2022).

Numerous studies have reported the isolation of *V. parahaemolyticus* resistant to antibiotics such as ampicillin and cephalothin (Silva et al. 2018), ampicillin, cefazolin, and penicillin (Tan et al. 2020), ampicillin, gentamicin, tetracycline, and fluoroquinolones (Lei et al. 2020), ampicillin, ampicillin/sulbactam, cefuroxime, cefoxitin, ceftazidime, cefepime, and colistin (Coutinho et al. 2019), cephalothin, cefoxitin and ceftazidime (da Silva et al. 2021), imipenem (Lee et al. 2018), amoxicillin/clavulanate, cefoxitin, cefotaxime, ceftazidime, cefuroxime, cefepime and trimethoprim/sulfamethoxazole in a New Delhi metallo-β-lactamase (bla_{NDM})-positive isolate (Briet et al. 2018), ampicillin, cefpodoxime, cefotaxime, ceftizoxime, tetracycline, ceftriaxone, ciprofloxacin and nalidixic acid (Parthasarathy et al. 2021).

V. vulnificus is susceptible to most antimicrobial agents such as β-lactams, third-generation cephalosporins, carbapenems, aminoglycosides, tetracyclines, fluoroquinolones, trimethoprim-sulfamethoxazole, and chloramphenicol. Nevertheless, the antimicrobial resistance in this pathogen is gradually increasing, with reports emerging on the isolation of strains resistant to clinically employed antibiotics such as ampicillin, doxycycline, tetracycline, aminoglycosides, macrolides, ciprofloxacin, amoxicillin, imipenem, aztreonam, and cephalosporins (Han et al. 2007; Baker-Austin et al. 2009; Elmahdi et al. 2016).

Mechanisms of antimicrobial resistance in *Vibrio* spp.

Early reports suggested the presence of a transmissible resistance (R) factor in *V. cholerae* (Prescott et al. 1968; Rahal et al. 1973; Hedges and Jacob 1975; Yokota and Kuwahara 1977). Subsequent studies on the antibiotic susceptibility of *V. cholerae*, a self-transmissible 62-kb, chromosomally integrating genetic element called the SXT element, which carried resistance genes to sulfamethoxazole, trimethoprim, and streptomycin was found in serotype O139 (Waldor et al. 1996). The genetic mechanisms of antimicrobial resistance identified in clinical and environmental isolates of *V. cholerae* include mobile genetic elements such as plasmids, transposons, integrons, and integrating conjugative elements (ICEs) (Verma et al. 2019; Das et al. 2020). *V. cholerae* O1 El Tor strains isolated from outbreaks in

Siberia and the Russian Far East carried SXT/R391 ICEs and genes such as *strA, strB* (streptomycin), *catB9/floR* (chloramphenicol/ florfenicol), *sul2* (sulfonamide), *dfrA1/ dhfR* (trimethoprim, and cotrimoxazole), and *tet(A)* (tetracycline) (Gladkikh et al. 2020). The multidrug-resistant (MDR) *V. cholerae* El Tor Biotype Ogawa serotype isolates from the 2010 outbreak in Odisha, India, carried elements like class I integron, SXT element, *aadA2* (aminoglycoside resistance), and *dfrA1* (trimethoprim resistance) genes (Jain et al. 2016). The whole genome sequence (WGS) of an MDR *V. cholerae* strain contained genetic elements coding for resistance to multiple antibiotics such as sulfonamide (*sul2*), trimethoprim (*dfrA1*), chloramphenicol (*catB9*), florfenicol (*floR*), and tetracycline [*tet(34)*] (Mevada et al. 2023). Novel genomic islands identified in *V. cholerae* non-O1/non-O139 carried mobile genetic elements such as Class 1 integrons and quinolone resistance determinants (Morita et al. 2020).

The β-lactam genes such as $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{SHV} have been detected in *Vibrio* isolates recovered from bivalves sold in retail markets (Dahanayake et al. 2020). The ESBL (extended-spectrum β-lactamase) genes $bla_{\text{CTX-M}}$ (87.5%), bla_{TEM} (40.6%), and bla_{SHV} (21.8%) were reported in *Vibrio* isolates from raw mussels in Korea (Hossain et al. 2020; Dahanayake et al. 2020). In another study, the β-lactamase encoding bla_{SHV} genes were detected in *Vibrio* isolates from water samples coast off North-East Sardina. At the same time, none of the *Vibrio* spp. harbored the bla_{TEM} gene (Zanetti et al. 2001). In India, the bla_{TEM} gene in *Vibrio* isolates from estuaries, shrimp farms, and seafood samples has been reported (Silvester et al. 2019). While the $bla_{\text{CTX-M}}$ was not detected in any isolates, the bla_{NDM} gene was found in 13.3% of the strains. A recent study made the first report of plasmid-encoded colistin resistance *mcr*-1 in a virulent strain of *V. parahaemolyticus* isolated from a shrimp sample in Hong Kong, China (Lei et al. 2019). The isolate carried bla_{CARB17} and quinolone resistance gene q*nrVC5*. Ampicillin resistance is widely prevalent in clinical and environmental isolates of *V. parahaemolyticus*, which, in addition to the production of β-lactamases, has been attributed to decreased synthesis of penicillin-binding proteins (Meng et al. 2023).

The quinolone resistance-determining regions (QRDR) of *gyrA* and *parC* and the plasmid-mediated quinolone resistance (PMQR) genes have been reported from fluoroquinolone-resistant *V. parahaemolyticus* with Ser-83-Ile, Ser-83-Phe substitutions in GyrA, and a Ser-85-Leu substitution in ParC (Lei et al. 2020). A novel class of chloramphenicol acetyltransferase, type C CAT or CATC, encoded by the *catC* gene identified in *V. parahaemolyticus*, confers varying levels of intrinsic resistance to chloramphenicol (Zhang et al. 2019). Recent studies have reported the occurrence of the bla_{NDM}-harboring *V. parahaemolyticus* and *V. vulnificus* strains from seafood and the environment (Briet et al. 2018; Oyelade et al. 2018). The bla_{TEM} and bla_{CMY} genes are also found in *V. parahaemolyticus* and *V. vulnificus* isolates (Oyelade et al. 2018). The WGS of NDM-positive *V. parahaemolyticus* revealed the presence of multiple antibiotic resistance genes such as *sul1, sul2, dfrA16, strA, strB* and *aadA2, floR* and *tet(A)* (Briet et al. 2018). A study showed the multiple mediators of tetracycline resistance in *V. parahaemolyticus* involving five different types of *tet* genes, viz. *tet(34), tet(A), tet(B), tet(M),* and *tet(E)* (Ye et al. 2023). Three different mutations (Q513K, S522L, and H526Y) in the *rpoB* gene of *V. vulnificus* have been attributed to high resistance to rifampicin (Cutugno et al. 2020). The ESBL-producing *V. vulnificus* isolated from imported seafood harbored a plasmid encoding ISE*c*9 upstream of the $bla_{\text{CTX-M-55}}$ and *qnrS2* genes (Nakayama et al. 2023).

Antimicrobial efflux pumps

The known species of *Vibrio* are resistant to multiple structurally distinct antimicrobial agents using a variety of disparate solute transporter systems. The transport of antimicrobial solutes across the membranes of living cells, including those of *Vibrio* spp., is conferred by integral membrane proteins, collectively called solute transporters (Stein 2012). Passive transport systems do not use biological energy to drive the passage of solutes and water across the biological membrane of the Vibrios (Sten-Knudsen 1978). Such transport mechanisms are driven by the energy of the solute or substrate gradients across the membrane. Active solute transporters, however, are driven by the energy stored in ATP released upon hydrolysis, as seen in primary active transport (Stein 1986), and the release of energy inherent in the membrane gradients of ions, as documented in secondary active transport, or co-transport, systems (West 1980; Stein 1986).

The efforts from the laboratory of Saier and colleagues led to the establishment and maintenance of the transporter classification database (TCD) https://www.tcdb.org/ (Saier et al. 2021). The TCD platform compiles transporters based on their functional properties and phylogenetic relationships between groups of homologous and related proteins. In general, transporters in living cells have been grouped into large superfamilies harboring sub-families with members that share similarities in sequence and structure, Fig. 1. Solute transporters are often evolutionarily conserved across all living taxa (Morita and Li 2016). The membrane transport proteins of *Vibrio* species fall into several large superfamilies (Table 1, Fig. 1). The ATP-binding cassette (ABC) superfamily encompasses the primary active transporters that utilize the hydrolysis of ATP to mediate the "uphill" transport of solutes across the membrane (Huda et al. 2003; Stephen et al. 2023). The efflux pumps of the secondary active transport type belong to the major facilitator superfamily

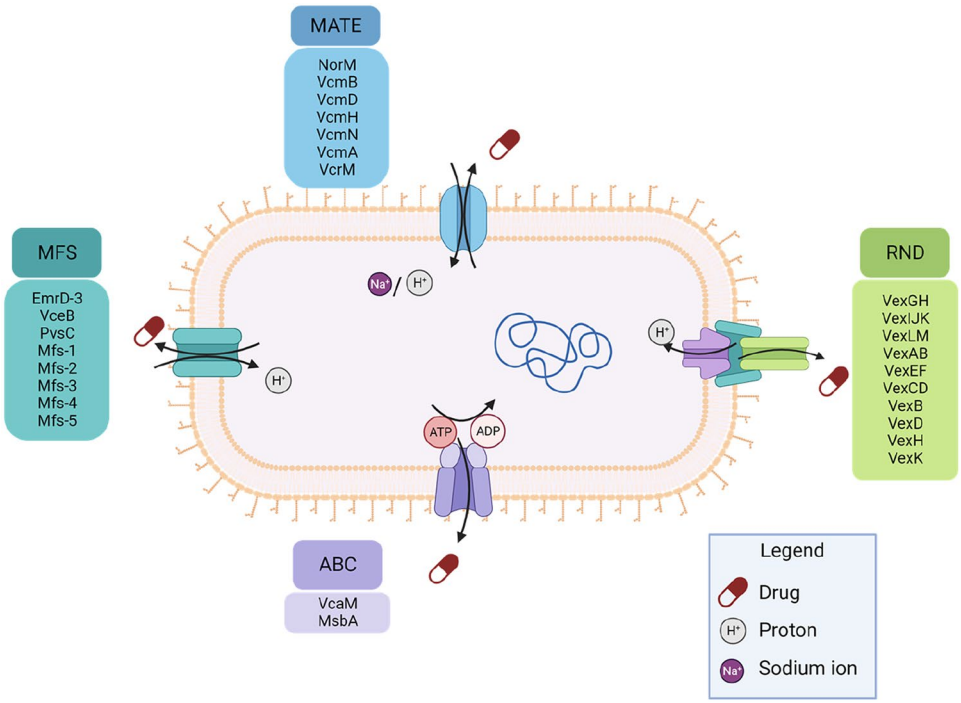

Fig. 1 Efflux proteins belonging to different superfamilies/families in *Vibrio* spp. The ABC superfamily of efflux pumps are primary active transporters energized by hydrolysis of ATP. In contrast, all other groups of proteins belong to the secondary active transporters, which utilize an electropotential gradient of H^+/Na^+ (cations) across the membrane to power the movement of solutes

(MFS), the resistance-nodulation-cell division (RND) superfamily, and the multidrug/oligosaccharidyl- lipid/polysaccharide (MOP) superfamily, the proteins of which function to extrude diverse solutes/substrates out of the cell and play critical roles in the antimicrobial resistance, virulence, and environmental persistence of *Vibrio* spp.

The multidrug/oligosaccharidyl-lipid/polysaccharide (MOP) superfamily of exporters includes members of the multidrug and toxic compound extrusion (MATE) (Kuroda and Tsuchiya 2009; Upadhyay et al. 2019; Claxton et al. 2021), the prokaryotic polysaccharide transporter (PST), the oligosaccharidyl-lipid flippase (OLF) from eukaryotes, and the mouse virulence factor (MVF) families of proteins (Hvorup et al. 2003). Likewise, the drug/metabolite transporter (DMT) superfamily contains related members of other transporter families, like the small multidrug resistance (SMR) family (Jack et al. 2001). The SMR family members QacEΔ1 and QacH have been studied in various *V. cholerae* non-O1 and *V. parahaemolyticus* isolates and have been shown to confer resistance to several quaternary ammonium compounds (Kazama et al. 1999; Ceccarelli et al. 2006). The SMR transport proteins have about 110 amino acids and four transmembrane domains, and they are postulated to oligomerize to form fully intact drug transporters (Jack et al. 2001).

The SMR and rhamnose transporter (RhaT) families from bacteria and the triose phosphate transporter (TPT) and the nucleotide-sugar transporter (NST) families from organelles of eukaryotic organisms and are discussed in detail elsewhere (Jack et al. 2001). More recently, the proteobacterial antimicrobial compound efflux (PACE) superfamily of proteins was discovered, harboring many multidrug efflux pumps energized by ion/substrate antiport, most uncharacterized (Hassan et al. 2021).

The major facilitator superfamily (MFS) consists of members that are passive and secondary active transporters from bacteria to humans (Henderson 1990, 1993; Griffith et al. 1992). The proteins of the resistance-nodulation-cell division (RND) class of secondary active transporters confer efflux of antimicrobial agents in bacteria, especially from the cells of the *Vibrio* genus of bacteria (Bina and Bina 2023). Together, the MFS and the RND transporters represent the most well-studied transporters of *V. cholerae*. This review article focuses on recent findings regarding the MFS, RND, and MATE transporters.

RND efflux pumps and their role in antimicrobial resistance and virulence

The RND efflux pumps are indispensable to the physiology of *Vibrio* spp. and play a critical role in their environmental persistence and pathogenesis (Stephen et al. 2022). *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus* genomes encode six, 12, and 11 RND efflux pumps, respectively (Taylor et al. 2012; Liu et al. 2022; Stephen et al. 2022). The unique structural architecture of the RND pumps consists of a tripartite protein assembly consisting of an outer membrane protein (OMP) and an inner membrane protein (IMP) connected by a periplasmic membrane

Table 1 Antimicrobial transporters, substrates, superfamilies, and transmembrane segment numbers in transporters of *Vibrio* spp.

Superfamily and members	TMS	Substrates	References
MFS			
EmrD-3	12	Multidrug	Smith et al. (2009)
VceB	14	Multidrug	Colmer et al. (1998)
PvsC	12	Vibrioferrin	Tanabe et al. (2003, 2006)
Mfs-1	12	Tetracycline, bile salts	Chen et al. (2013)
Mfs-2	12	Tetracycline, bile salts	
Mfs-3	14	Tetracycline, bile salts	
Mfs-4	12	Tetracycline, bile salts	
Mfs-5	12	Tetracycline, bile salts	
RND			
VexGH	12	Multidrug	Tseng et al. (1999), Kunkle et al. (2017)
VexIJK	12	No known substrates	
VexLM	12	No known substrates	
VexAB	12	Multiple antimicrobials, Dyes and detergents	
VexEF	12	Multiple antimicrobials, Dyes and detergents	
VexCD	12	Cholate	Tseng et al. (1999), Rahman et al. (2007)
VexB	12	Multiple antimicrobials, Detergents, and bile salts	Taylor et al. (2012)
VexD	12	Bile salt	
VexH	12	Ampicillin and Novobiocin	
VexK	12	Detergent, Novobiocin	
MATE (MOP)			
NorM	12	Multidrugs	Morita et al. (2000)
VcmB	12	Fluoroquinolones, ethidium bromide, Hoechst 33,342	Hvorup et al. (2003), Begum et al. (2005)
VcmD	12	Fluoroquinolones, Aminoglycosides, Ethidium Bromide, Hoechst 33,342	
VcmH	12	Fluoroquinolones, Aminoglycosides, ethidium bromide, Hoechst 33,342	
VcmN	12	Fluoroquinolones, ethidium bromide, Hoechst 33,342	
VcmA	12	Fluoroquinolones, ethidium bromide, Hoechst 33,342	
VcrM	12	Ethidium bromide, Hoechst 33,342	
ABC			
VcaM	6	Multidrugs	Lu et al. (2018)
MsbA	6	Lipid A, Lipopolysaccharides, multidrugs	Chang (2003)

fusion protein (MFP) (Fig. 2). The entire setup is organized into a continuous transmembrane channel, allowing the movement of substrates in an inward-outward fashion (protoplasm to the outside) without entering the periplasmic space (Nikaido 1994, 2011; Destoumieux-Garzón et al. 2014; Symmons et al. 2015). The antiport activity is vested with the inner membrane protein (IMP) energized by proton (H^+) gradients. Although this membrane channel is expected to transport lipophilic substrates preferentially, the RND efflux pumps are generally non-specific and allow the extrusion of diverse substrates of all charge types (Nikaido 1998, 2011). As an exception to all known RND pumps, VexEF is energized by the electrochemical gradient of Na^+ ions instead of H^+ (Rahman et al. 2007).

The Vex group of the RND efflux pumps (VexAB, VexCD VexEF, VexGH, VexIJK, VexLM) from *V. cholerae* O1 are a group of bile-regulated proteins that confer resistance to bile and certain antimicrobial compounds. A whole-genome comparison of a non-O1 *V. cholerae* strain PS15 with *V. cholerae* O1 strain N16961 identified all of these efflux pumps except VexE (Mukherjee et al. 2014). The *vexAB*, *vexCD*, *vexEF*, *vexGH*, and *vexIJK* elements are on the large chromosome. At the same time, the *vexLM* gene is located on the small chromosome. All are organized into operons with an MFP-encoding gene upstream of the efflux pump (IMP) gene, and the operon does not contain the OMP-encoding gene (Bina and Bina 2023). Since bile acids are antimicrobial, bile resistance is necessary for successful

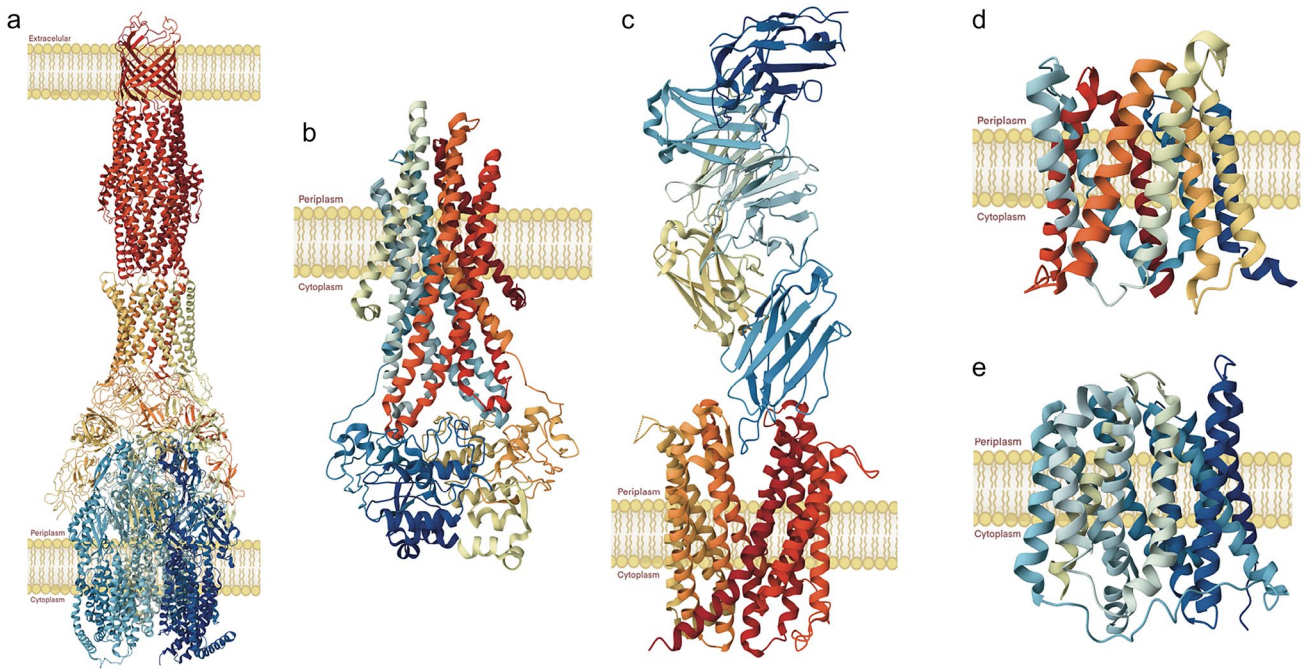

Fig. 2 Transport proteins from *Vibrio* bacteria. Depicted are known protein structures from species of the *Vibrio* genus, representative examples of which include **a** AcrAB-TolC of *V. vulnificus* (PDB 5V5S), **b** MsbA from *V. cholerae* (PBD 5TTP), **c** NorM (PDB 7PHP) from *V. cholerae*, **d** YddG, (PDB 5I20) a known DMT protein that is homologous to a putative permease from *V. vulnificus*, and **e** EmrD (PDB 2GFP), which is homologous to EmrD-3 of *V. cholerae*

intestinal colonization by a pathogenic bacterium such as *V. cholerae*. Early insights into the role of RND efflux pumps in bile resistance suggested the functional role of VexAB in intrinsic bile resistance in *V. cholerae* O1. At the same time, VexCD complemented this function of VexAB (Bina et al. 2006). The importance of RND efflux pumps in bile and antimicrobial resistance has been demonstrated using deletion mutants of *V. cholerae*. *V. cholerae* El Tor strain N16961 lacking all six RND efflux pumps was hypersensitive to bile, detergents, and antibiotics, produced significantly less cholera toxin and TCP protein, and was unable to colonize infant mouse intestines, suggesting the synergistic role of RND efflux pumps in the physiology and pathogenesis of *V. cholerae* (Bina et al. 2008).

Further, the absence of RND-mediated efflux resulted in the accumulation of metabolites in the periplasmic space, leading to the activation of periplasmic sensors involving two-component systems and the ToxR regulator as an adaptive response (Bina et al. 2018). This study conclusively showed the role of RND efflux pumps in regulating the expression of ToxR-mediated virulence factors, including TCP and the cholera toxin. However, this mechanism involves a complex network of proteins responding to stress in *V. cholerae* (Bina et al. 2018). Another study demonstrated the role of VexGH in Vibriobactin secretion in *V. cholerae*. Deleting the *vexGH* locus impaired homeostasis and reduced fitness under iron-limiting conditions (Kunkle et al. 2017; Du et al. 2018).

Without an identified OMP-encoding gene within the *vex* operon, researchers attempted to express *V. cholerae* Vex RND proteins in *E. coli* with the TolCvc outer membrane factor from *V. cholerae* non-O1 (Rahman et al. 2007). The VexAB and VexCD components could function using $TolC_{vc}$ and confer elevated MICs of cholates. VexAB-$TolC_{vc}$ also increased the MICs of erythromycin, novobiocin, rhodamine 6G, and TPPCl (Rahman et al. 2007). Similarly, VexEF-$TolC_{vc}$ was functional in an *E. coli* background and could efflux ethidium bromide in the presence of Na^+. No definite antimicrobial substrate has been reported for the VexEF and VexLM pumps. However, VexEF showed efflux activity for bile, detergents, erythromycin, and novobiocin in an *E. coli* background (Rahman et al. 2007; Taylor et al. 2012; Bina and Bina 2023).

The efflux pumps homologous to VexAB, VexCD, and *E. coli* AcrAB (Fig. 2) encoded in the *V. vulnificus* genome have been shown to alter the bacterium's antimicrobial susceptibilities. Increased susceptibility to erythromycin, ethidium bromide, acriflavine, and bile salts was observed in VexAB deletion mutants. Further, increased susceptibility was seen only to acriflavine in an AcrAB homolog mutant. No change in antimicrobial susceptibility was observed in a VexCD homolog deletion mutant,

suggesting that these efflux pumps differ substantially in their preferred substrates and might contribute to other important physiological activities of the bacterium needing further investigation (Lee et al. 2015).

Twelve RND pumps of *V. parahaemolyticus* have been cloned in a hypersensitive *E. coli* background and characterized (Matsuo et al. 2007, 2013). Although VmeAB conferred significantly higher MICs of different antimicrobial agents in *E. coli*, the mutant strain of *V. parahaemolyticus* lacking VmeAB did not show the same resistance levels. However, a slight increase in the MICs was observed (Matsuo et al. 2007). In contrast, other efflux pumps, VmeCD, VmeEF, and VmeYZ, showed higher MICs in *E. coli,* and the effect was similar in deletion mutants of these efflux pump-encoding genes (Matsuo et al. 2013). Further, VmeAB, VmeCD, VmeEF, and VmeYZ co-expressed with VpoC, an outer membrane component in *V. parahaemolyticus* in *E. coli*, exhibited significantly higher MICs of various antimicrobials compared to the same efflux pumps functional with TolC of *E. coli* (Matsuo et al. 2013). VmeGHI, VmeJK, VmeLM, and VmeTUV were functional and could confer higher MICs only with VpoC. A null mutant lacking all 12 RND efflux pumps was highly sensitive to various antimicrobials. It showed a significantly lower fluid accumulation in rabbit ileal loops, suggesting that RND efflux pumps in *V. parahaemolyticus* contribute to its pathogenicity. These RND efflux pumps function to resist deoxycholates and other antimicrobials, are involved in the production or transport of siderophore vibrioferrin, and contribute to the pathogenicity of *V. parahaemolyticus*.

Proteomic profiling of the integral membrane proteins in *V. parahaemolyticus* revealed the involvement of the periplasmic adaptor subunit and the permease subunit of the RND efflux pump in virulence (Pérez-Acosta et al. 2018). The deletion of a membrane fusion protein, VmeL, belonging to the RND efflux pumps of *V. parahaemolyticus* resulted in lowered cytotoxicity towards HeLa cells, reduced virulence in a murine intraperitoneal infection assay, and an absence of lateral flagella hindering the motility (Liu et al. 2022). A *ΔvmeL* transcriptome analysis revealed a downregulation of genes related to the Type III Secretion 1 system (Liu et al. 2022), an important virulence factor involved in adhesion to the host cell (Wu et al. 2020; Liu et al. 2022). Similar studies in *V. vulnificus* involving the deletion of three RND efflux pumps VexBHZ showed increased cytotoxicity and biofilm formation (Lo et al. 2017). Analysis of whole genome sequences of *Vibrio* spp. can help identify new RND-type efflux pumps contributing to antimicrobial resistance (Lloyd et al. 2019).

MATE efflux pumps

The MATE group of ion/drug antiport systems is widely encountered in *Vibrio* spp. as well as in other Gram-negative and -positive bacteria. The first MATE-type transporter to be described was NorM from *V. parahaemolyticus* (Morita et al. 2000). Subsequently, proteins belonging to this group were reported from all domains of life performing essential tasks of detoxification and maintaining cellular homeostasis. These proteins actively extrude cationic substrates, including xenobiotics, drugs, toxic metabolites, hormones, and organic acids in plants (Kusakizako et al. 2020). The MATE efflux proteins fold into 12 transmembrane helices similar to the MFS transporters (Fig. 2). However, the topology of MATE proteins is distinctly different from the MFS family of efflux proteins, suggestive of different evolutionary origins of these two groups of membrane proteins (He et al. 2010; Kusakizako et al. 2020). Homologous MATE-type efflux pumps in other bacteria are NorM-VC, VcmA, and VcrM of *V. cholerae* (Huda et al. 2003), YdhE of *Escherichia coli* (Brown et al. 1999; Morita et al. 2000), BexA of *Bacteroides thetaiotaomicron* (Miyamae et al. 2001), PmpM from *P. aeruginosa* (He et al. 2004), NorM-NG of *Neisseria gonorrhoeae* (Rouquette-Loughlin et al. 2003), AbeM of *Acinetobacter baumannii* (Su et al. 2005), NorM of *Erwinia amylovora* (Burse et al. 2004), and KetM of *K. pneumoniae* (Ogawa et al. 2015). These transporters extrude cationic compounds such as DNA-intercalating dyes, detergents, aminoglycoside, and fluoroquinolone antibiotics. Although a majority of these are Na^+-dependent, efflux pumps of this category either use H^+ and Na^+ simultaneously or H^+ alone. For example, *V. cholerae* NorM can simultaneously couple with Na^+ and H^+, while AbeM of *Acinetobacter baumannii* is uniquely H^+-dependent (Su et al. 2005; Jin et al. 2014). Similarly, VcmB, VcmD, and VcmH efflux pumps are Na^+-dependent, while VcmN is Na^+-independent (Begum et al. 2005). The homologous MATE efflux pumps are grouped into three clusters (Brown et al. 1999). Cluster 1 contains NorM of *V. parahaemolyticus* (NorM-VP), *V. cholerae* (NorM-VC), and YdhE of *E. coli*, while DinF of *E. coli* and its homologues such as VcmN of *V. cholerae* and PfMATE of *Pyrococcus furiosus* are in cluster 3 (Tanaka et al. 2013; Ogawa et al. 2015). Cluster 2 consists of eukaryotic MATE-type transporters. Mutational analyses have revealed that Asp-371 of NorM from *V. cholerae* is essential for substrate transport, and this residue is widely conserved across the Na^+-dependent MATE transporters of cluster 1 (Otsuka et al. 2005; Ogawa et al. 2015). The cation-bound crystal structure of NorM-VC showed two amino acid residues, Glu-255 and Asp-371, located at the center of C-terminal

lobes formed by TM7-12 constitute the substrate-binding pockets (He et al. 2010; Kusakizako et al. 2020). (Otsuka et al. 2005) showed that Asp-32, Glu-251, and Asp-367 in NorM-VP play important roles in the Na^+-dependent drug transport process. Asp-367 in NorM-VP corresponds to Asp-371 of NorM-VC, and the replacement of Asp-367 with either alanine or asparagine severely impaired the drug transport activity of NorM-VP (Otsuka et al. 2005).

Lu and colleagues determined the crystal structure of Na^+-coupled NorM-NG from *Neisseria gonorrhoeae* bound to three different substrates. They showed that Asp-41, Phe-265, Gln-284, Asp-355, and Asp-356 are essential for transport function, and these are supposedly conserved across prokaryotic and eukaryotic MATE-type transporters (Lu et al. 2013). Na^+ and substrate bind distinct amino acids in NorM and bind simultaneously, in contrast to the general notion that Na^+ and the substrate bind at two different stages corresponding to two different conformational statuses of the efflux protein (Lu et al. 2013). Asp-377 in NorA-NG and its corresponding residues Asp-367 and Asp-371 in NorA-VP and NorA-VC, respectively, have been shown to be critically important for substrate transport (Otsuka et al. 2005; Lu et al. 2013).

The crystal structure of another H^+/drug antiport protein from *V. cholerae*, VcmN, and the subsequent molecular simulation studies have provided significant insights into the dynamics of ion coupling in this as well as other proteins of the DinF subfamily (Kusakizako et al. 2020; Castellano et al. 2021; Claxton et al. 2021). VcmN, originally proposed to be an H^+/drug antiport with water bound to the N-lobe cavity, was later shown to exhibit Na^+-dependent changes in conformation, suggesting that it could be Na^+-driven like a majority of other MATE transporters (Kusakizako et al. 2020; Castellano et al. 2021; Claxton et al. 2021).

Two MATE efflux pumps of H- and D-type characterized from clinical isolates of *V. fluvialis* were dependent on Na^+/K^+ ions, and of these, the H-type pump extruded fluoroquinolones, ethidium bromide, and safranin, while the D-type extruded ethidium bromide only (Mohanty et al. 2012, 2021). Interestingly, unlike other MATE-type transporters, the H- and D-type pumps of *V. fluvialis* are made up of 10 and 11 TMS, respectively. However, molecular docking studies revealed a similar transport mechanism to NorM, with an aspartic acid residue in TM-1 acting as an ion binding site (Mohanty et al. 2021).

MFS efflux pumps

The multidrug efflux pumps of the MFS constitute a large group of related transporters from all known taxa (Pao et al. 1998; Saier et al. 1999). The MFS proteins share conserved sequences and structures, suggesting they share similar transport mechanisms across the membrane (Griffith et al. 1992; Varela and Griffith 1993). These transport systems confer resistance to antimicrobial and anticancer agents (Kumar et al. 2020; Hou et al. 2022). As such, these integral membrane proteins constitute suitable targets for modulation to restore the efficacy of chemotherapy for recalcitrant infections and cancer (Kumar et al. 2013b, 2016a; Stephen et al. 2022; Dhakne et al. 2023).

MFS efflux pumps of *Vibrio* spp.

According to the complete genome sequence of the *V. cholerae* O1 biovar El Tor toxigenic strain N16961, 18 genetic elements are thought to encode major facilitator superfamily transporters (Heidelberg et al. 2000). Several putative efflux pump-encoding genes from *Vibrio* spp. have been characterized physiologically (Stephen et al. 2022). The first MFS multidrug efflux pump from *V. cholerae* to be cloned, isolated, and studied for antimicrobial resistance is VceB, an integral membrane protein with 14 predicted transmembrane (TM) helices (Colmer et al. 1998). The *vceB* gene is encoded on the *V. cholerae* genome as part of the VceCRAB operon, where VceA and VceB are homologous to *Escherichia coli* proteins EmrA and EmrB, respectively (Lomovskaya and Lewis 1992). The VceCRAB locus encodes a multi-component membrane protein that effluxes multiple antimicrobial agents. VceC is considered an outer membrane protein by its functional similarity to OprM from *Pseudomonas aeruginosa* (Bai et al. 2010). VceR plays a functional role as a transcriptional regulator (Alatoom et al. 2007). VceA is a periplasmically-located membrane-fusion protein (Woolley et al. 2005). Therefore, the VceCRAB regulon encodes a tripartite antimicrobial efflux system consisting of VceC, VceA, and VceB (Woolley et al. 2005; Alatoom et al. 2007; Bai et al. 2010).

The second MFS multidrug efflux pump system discovered in *V. cholerae* is EmrD-3, physiologically studied in our laboratory (Smith et al. 2009). We found the *emrD-3* gene on the chromosome of an O395 *V. cholerae* (Kumar et al. 2013a). The predicted structure of EmrD-3 encases another integral membrane protein, largely hydrophobic but with 12 TMs (Smith et al. 2009). We found that EmrD-3 confers transport of ethidium bromide across the membrane in a proton-driven manner and confers elevated resistance to multiple structurally distinct antimicrobial agents, such as linezolid, rifampin, erythromycin, and chloramphenicol, among others. Our laboratory performed a genomic comparative study of the *emrD-3* gene from O395 with a toxigenic strain of *V. cholerae* N16961 and environmental isolate from Puget Sound, a strain denoted as PS15 (Kumar et al. 2013a; Mukherjee et al. 2014). We observed that *emrD-3* was located on chromosome II of *V. cholerae* N16961 but absent

from *V. cholerae* PS15 (Mukherjee et al. 2014). Due to the presence of EmrD-3 in toxigenic *V. cholerae* but missing in non-toxigenic counterparts, we anticipate that this multidrug efflux pump is a suitable target for antimicrobial resistance modulation to restore and enhance clinical treatment of severe cholera infections (Kumar et al. 2016a). Toward this, our laboratory discovered that both antimicrobial resistance levels and membrane drug transport activities by EmrD-3 are reduced by extracts of *Allium sativum* (garlic) and its pure bioactive component, allyl sulfide (Bruns et al. 2017). In the same study, we observed that *A. sativum* extract synergistically affects the *V. cholerae* growth with multiple antimicrobial agents.

From *V. parahaemolyticus*, the MFS transporter PvsC was discovered, showing the export of the iron siderophore vibrioferrin (Tanabe et al. 2003). This system is thought to enhance microbial virulence. The gene for PvsC is encoded on the *pvsABCDE* operon, and it is thought to play a role in the metabolism and efflux of *Vibrio*-specific siderophores (Tanabe et al. 2006).

Another set of MFS transporters from *V. cholerae* denoted as Mfs-1 through Mfs-5, five determinants totally, lose resistance to tetracycline and bile when the ORFs are deleted by mutation (Chen et al. 2013). The same study observed that expression programs of the *mfs1-5* genes are controlled by a protein homologous to members of a family of well-characterized transcriptional regulators, namely LysR (Maddocks and Oyston 2008).

Role of efflux pumps in the physiology of *Vibrio* spp.

Using the deduced primary sequence (amino acid) of EmrD-3 as a query sequence in genomic databases, we found homologs in distantly related bacteria, like *Proteus mirabilis* and *Bacillus cereus*, but also in other species of the *Vibrio* genus, such as *V. harveyi*, *V. vulnificus*, and *V. parahaemolyticus*, indicating that these bacteria harbor conserved MFS multidrug resistance-conferring transporters as putative virulence factors (Smith et al. 2009; Mukherjee et al. 2014; Stephen et al. 2022).

Interestingly, the MFS VceB, EmrD-3, and Mfs1-5 transporters harbor elements of highly conserved sequence motifs, such as motif A, present on the loop between helices two and three of virtually all members of the MFS, and the antiporter motif, also called motif C, that resides in the fifth helix of MFS drug and multidrug efflux pumps (Henderson 1990; Varela et al. 1995; Colmer et al. 1998). The functional roles conferred by motifs A and C residues have been extensively studied in transporters of the MFS (Kumar et al. 2016b; Kakarla et al. 2017).

The signature sequence of motif A, "Gly-$(X)_3$-Asp-Arg/Lys-X-Gly-Arg-Arg/Lys," is highly conserved in virtually all members of the MFS, attesting to the functional importance of its residues in a variety of solute transporters from all living species including multidrug efflux pumps from known species of *Vibrio* bacteria (Griffith et al. 1992, 1994; Varela and Griffith 1993; Stephen et al. 2022). Thus, the homologous nature of the MFS transporters strongly suggests that molecular physiological studies of proteins from various species apply to the MFS transporters from the *Vibrio* genus. Structure–function studies of residues belonging to motif A have demonstrated critical roles in bacterial tetracycline efflux pumps, such as antimicrobial binding in TetA(C) of *E. coli* (McMurry et al. 1980), the substrate channel pathway (Yamaguchi et al. 1992), and elements of the transporter gate in TetA(B) (Someya et al. 2000). Residues of motif A were demonstrated to play important roles in stabilizing the transporter structure by salt-bridge formation in the YajR efflux pump of *E. coli* (Jiang et al. 2013). In various MFS transporters, motif A residues are proposed to mediate an essential role in conformational changes during substrate transport, such as in the LacY lactose symporter of *E. coli* (Varela and Wilson 1996). The LmrP drug transporter of *Lactococcus lactis*, with the protonation of acidic residues, influences a conformational switching system (Masureel et al. 2014). In the same study, the structure formed by residues of motif A appears to detect the energy status of the respiratory chain. This energy-sensing system should be relevant to the secondary active transporters of the MFS.

The laboratories of Skurray and Henderson identified a highly conserved consensus sequence in MFS efflux pumps that operate by an antiport process (Henderson 1990; Rouch et al. 1990). The signature sequence is "Gly-$(X)_8$-Gly-$(X)_3$-Gly-Pro-$(X)_2$-Gly-Gly," called the "antiporter motif" and later motif C (Varela and Griffith 1993; Varela et al. 1995; Ginn et al. 2000). Interestingly, when multiple amino acid sequence alignments were performed manually, the motif C appeared in symporters of the MFS, implicating a critical role for this conserved sequence in most proteins of the MFS (Yaffe et al. 2013). We showed by mutational analysis that the highly conserved glycine residue of the Gly-Pro dipeptide confers antimicrobial resistance in the TetA(C) tetracycline efflux pump encoded on the cloning plasmid pBR322 in host *E. coli* (Varela et al. 1995). The functional importance of the Gly-Pro dipeptide was demonstrated in TetA(L) and TetA(K) tetracycline efflux pumps from *Staphylococcus aureus* and *Bacillus subtilis* and shown to form a barrier to proton leakage, thus preventing extraneous ion-substrate energy coupling (Konishi et al. 1999; Jin and Krulwich 2002; De Jesus et al. 2005). Residues of motif C were shown to mediate conformational changes during the transport cycle of TetA(K) (Ginn et al. 2000), possibly influencing the direction of antimicrobial transport as predicted

(Maiden et al. 1987; Pao et al. 1998; Saier et al. 1999). In the QacA multidrug efflux pump of *S. aureus*, residues of motif C bind antimicrobial substrates as they are translocated across the membrane (Hassan et al. 2006). An analysis of the structure–function relationship regarding motif C revealed its participation in forming a centrally-located substrate-binding cavity in the CaMdr1p drug transporter of antifungal agents from cells of *Candida albicans*, a eukaryote (Pasrija et al. 2007). Structurally speaking, in the mammalian vesicular monamine pump VMAT2, motif C forms an interface between the two large global bundles characteristic of the MFS transporters (Yaffe et al. 2013). Along these lines, residues of motif C form a molecular hinge structure that influences conformational changes during drug/ion antiport (Luo and Parsons 2010; Yaffe et al. 2013). Thus, the molecular structure formed by residues of motif C serves as a regulator of conformational switching during antimicrobial efflux in MFS transporters, a process relevant to virulence in cells of *Vibrio* species (Luo and Parsons 2010; Stephen et al. 2022).

Efflux pumps in biofilm formation and quorum sensing

The toxin-coregulated pilli (TCP) in *V. cholerae* is known to play a role in biofilm differentiation. The ΔTcpA (TCP pilin subunit) mutant in biofilms on the chitinous surface was undifferentiated, leading to high sensitivity to biocide like sodium dodecyl sulfate (SDS). Upon exposure to 0.2% SDS, the ΔTcpA mutant cells disassociated from the undifferentiated biofilm within 5 min, while the wildtype *V. cholerae* El Tor biofilm remained unaffected even after 30 min (Reguera and Kolter 2005). The RND efflux pump, like VexH in *V. cholerae,* is vital for the production of TCP (Taylor et al. 2012). Thus, the RND efflux pumps might also form biofilm on chitinous surfaces like copepods and zooplankton, which are associated with cholera outbreaks (Lipp et al. 2002). The biofilm formation capacity of *V. parahaemolyticus* was compromised upon deletion of VmeL, a membrane fusion protein belonging to the RND family of efflux pumps, conserved with more than 90% sequence similarity in *V. diabolicus, V. harveyi, V. alginolyticus, V. chemaguriensis,* and *V. rotiferianus* (Liu et al. 2022). The disruption of RND efflux pumps via deletion of the *tolC*-encoded component in *V. cholerae* leads to the activation of the family of LysR-type transcriptional regulator *leuO* (Weng et al. 2021); *leuO* is associated with biofilm formation in *V. cholerae* (Moorthy and Watnick 2005).

The relation between RND efflux pumps and quorum sensing (QS) has been investigated and proven in pathogens like *Pseudomonas aeruginosa* (Evans et al. 1998; Maseda et al. 2004), *Escherichia coli* (Rahmati et al. 2002), *Salmonella enterica* (Dawan et al. 2022), *Acinetobacter baumannii* (Abd El-Rahman et al. 2023). The QS molecules are known to act as a substrate for the efflux pumps, particularly those that cannot diffuse across the membrane passively (Rahmati et al. 2002; Piddock 2006). For *V. cholerae,* CqsA produces cholera autoinducer-1(CAI-1) (Wei et al. 2011), and LuxS produces autoinducer-2 (AI-2) (Bassler et al. 1993), known to play an essential role in virulence (Higgins et al. 2007) and biofilm formation (Hammer and Bassler 2003). A definitive part of RND efflux pumps in *V. cholerae* QS is yet to be established.

Conclusions

Cholera remains a serious healthcare concern on a global scale (Mandal et al. 2017). The causative agent of cholera, *V. cholerae*, and related species have acquired resistance to antimicrobial agents that are shared amongst them and unrelated microorganisms (Kitaoka et al. 2011). Of these resistance mechanisms, integral membrane transporters represent key multidrug resistance systems (Stephen et al. 2022). These bacterial multidrug efflux pumps make suitable targets for modulation to restore the clinical efficacy of antimicrobial agents in cases of severe infection (Kumar et al. 2016a; Stephen et al. 2022). Unfortunately, agents of resistance modulation are poorly understood, indicating that such studies are needed.

Future research programs can consider investigating new strategies for treating multidrug-resistant variants that cause severe disease (Varela and Kumar 2019). Thus, new modulators of antimicrobial efflux and other antimicrobial resistance mechanisms are needed. Putative modulators require translation to the clinical setting in regions with high cholera incidence and prevalence. Furthermore, studies of the physiological and molecular mechanisms of resistance modulation will provide new insights into the efficacy restoration of unrelated infections in which similar targets confound treatment.

Continued structure–function investigations are needed to fully understand the basic mechanisms of multidrug efflux in bacterial pathogens. For instance, the molecular mechanisms of energy transduction during primary and secondary active efflux pumps are not fully understood for antimicrobial efflux pumps in *Vibrio* species and related pathogens. Along these lines, a clear understanding is lacking of the relationships between the conformational changes that occur during transport and the mechanisms responsible for antimicrobial specificity. Interestingly, investigations of the highly conserved amino acid sequences shared by members of the various transporter superfamilies have provided insights into these mechanisms of solute translocation across the membrane. The MFS molecular hinge structure and its mode of operation during

transport appear to provide a universal mechanism of solute transport. This characteristic may be beneficial not only for our basic understanding of transporter physiology but also as a means of addressing the problem of clinical resistance to antimicrobial-resistant bacterial pathogens.

Another field of study that is seemingly neglected involves our understanding of the transporter mechanisms that dictate substrate specificity. Some transporters are specific for a narrow range of substrates, like the MFS efflux pumps for the tetracycline class of antimicrobials (Nelson and Levy 2011). In contrast, many transporters are relatively more promiscuous, harboring many structurally dissimilar substrates (Alvarez-Ortega et al. 2013; Delmar et al. 2014; Ranaweera et al. 2015). It is poorly understood how these disparate transporter systems determine whether a given transporter possesses a single drug class of substrate specificity versus those of multiple drugs while simultaneously preventing leakage of ions or water that would dissipate the energetic driving forces of primary and secondary active transport.

Lastly, more work needs to be conducted regarding the nature of the pathway of substrates through the various transporters. How much multidrug transporters can accommodate the structurally distinctive antimicrobial agents is unclear. At the molecular physiological level, it remains fascinating how individual multidrug transporters appear to have a list of unique substrates for each. This issue of antimicrobial specificity remains relevant in the clinical setting and is a focus of future investigation.

Author contributions MFV and SK initiated and organized the project. AO, MA, ML, SK, JS, and MFV collected literature and wrote distinct sections of the manuscript. SK and MFV compiled, edited, and prepared the final form of the manuscript. All authors reviewed and agreed to the published form of the article.

Funding The research studies reported from our laboratories and reflected in this publication were supported by Faculty Research and Instructional Development grants by ENMU and the National Institute of the General Medical Sciences (P20GM103451) awarded by the National Institutes of Health, plus the HSI-STEM program from the U.S. Department of Education (P031C110114).

Declarations

Conflict of interest The authors here declare no conflict of interest.

Consent for publication Not applicable.

Ethical approval Not applicable.

References

Abd El-Rahman OA, Rasslan F, Hassan SS et al (2023) The RND efflux pump gene expression in the biofilm formation of *Acinetobacter baumannii*. Antibiotics 12:419. https://doi.org/10.3390/antibiotics12020419

Ahmed HA, El Bayomi RM, Hussein MA et al (2018) Molecular characterization, antibiotic resistance pattern and biofilm formation of *Vibrio parahaemolyticus* and *V. cholerae* isolated from crustaceans and humans. Int J Food Microbiol 274:31–37. https://doi.org/10.1016/j.ijfoodmicro.2018.03.013

Alatoom AA, Aburto R, Hamood AN, Colmer-Hamood JA (2007) VceR negatively regulates the *vceCAB* MDR efflux operon and positively regulates its own synthesis in *Vibrio cholerae* 569B. Can J Microbiol 53:888–900. https://doi.org/10.1139/W07-054

Albert MJ, Siddique AK, Islam MS et al (1993) Large outbreak of clinical cholera due to *Vibrio cholerae* non-O1 in Bangladesh. Lancet 341:704. https://doi.org/10.1016/0140-6736(93)90481-u

Ali M, Nelson AR, Lopez AL, Sack DA (2015) Updated global burden of cholera in Endemic Countries. PLoS Negl Trop Dis 9:e0003832. https://doi.org/10.1371/journal.pntd.0003832

Alvarez-Ortega C, Olivares J, Martínez JL (2013) RND multidrug efflux pumps: what are they good for? Front Microbiol 4:7. https://doi.org/10.3389/fmicb.2013.00007

Archer EJ, Baker-Austin C, Osborn TJ et al (2023) Climate warming and increasing *Vibrio vulnificus* infections in North America. Sci Rep 13:3893. https://doi.org/10.1038/s41598-023-28247-2

Ayyappan MV, Balange AK, Nayak BB, Kumar S (2018) Distribution of potentially pathogenic *Vibrio parahaemolyticus* in seafood and the aquatic environment of Mumbai, India. Fish Technol 55:205–211

Bag PK, Bhowmik P, Hajra TK et al (2008) Putative virulence traits and pathogenicity of *Vibrio cholerae* Non-O1, Non-O139 isolates from surface waters in Kolkata, India. Appl Environ Microbiol 74:5635–5644. https://doi.org/10.1128/AEM.00029-08

Bai J, Mosley L, Fralick JA (2010) Evidence that the C-terminus of OprM is involved in the assembly of the VceAB-OprM efflux pump. FEBS Lett 584:1493–1497. https://doi.org/10.1016/j.febslet.2010.02.066

Baker-Austin C, McArthur JV, Lindell AH et al (2009) Multi-site analysis reveals widespread antibiotic resistance in the marine pathogen *Vibrio vulnificus*. Microb Ecol 57:151–159. https://doi.org/10.1007/s00248-008-9413-8

Baker-Austin C, Oliver JD, Alam M et al (2018) *Vibrio* spp. infections. Nat Rev Dis Primers 4:8. https://doi.org/10.1038/s41572-018-0005-8

Bassler BL, Wright M, Showalter RE, Silverman MR (1993) Intercellular signalling in *Vibrio harveyi*: sequence and function of genes regulating expression of luminescence. Mol Microbiol 9:773–786. https://doi.org/10.1111/j.1365-2958.1993.tb01737.x

Begum A, Rahman MM, Ogawa W et al (2005) Gene cloning and characterization of four MATE family multidrug efflux pumps from *Vibrio cholerae* non-O1. Microbiol Immunol 49:949–957. https://doi.org/10.1111/j.1348-0421.2005.tb03690.x

Bhattacharya K, Kanungo S, Sur D et al (2011) Tetracycline-resistant *Vibrio cholerae* O1, Kolkata, India. Emerg Infect Dis 17:568–569. https://doi.org/10.3201/eid1703.101176

Bina XR, Bina JE (2023) *Vibrio cholerae* RND efflux systems: mediators of stress responses, colonization and pathogenesis. Front Cell Infect Microbiol 13:1203487. https://doi.org/10.3389/fcimb.2023.1203487

Bina JE, Provenzano D, Wang C et al (2006) Characterization of the *Vibrio cholerae* vexAB and vexCD efflux systems. Arch Microbiol 186:171–181. https://doi.org/10.1007/s00203-006-0133-5

Bina XR, Provenzano D, Nguyen N, Bina JE (2008) *Vibrio cholerae* RND family efflux systems are required for antimicrobial resistance, optimal virulence factor production, and colonization of the infant mouse small intestine. Infect Immun 76:3595–3605. https://doi.org/10.1128/IAI.01620-07

Bina XR, Howard MF, Taylor-Mulneix DL et al (2018) The *Vibrio cholerae* RND efflux systems impact virulence factor production and adaptive responses via periplasmic sensor proteins. PLoS Pathog 14:e1006804. https://doi.org/10.1371/journal.ppat.1006804

Blevins SM, Bronze MS (2010) Robert Koch and the 'golden age' of bacteriology. Int J Infect Dis 14:e744–e751. https://doi.org/10.1016/j.ijid.2009.12.003

Bonnin-Jusserand M, Copin S, Le Bris C et al (2019) Vibrio species involved in seafood-borne outbreaks (*Vibrio cholerae*, *V. parahaemolyticus* and *V. vulnificus*): review of microbiological versus recent molecular detection methods in seafood products. Crit Rev Food Sci Nutr 59:597–610. https://doi.org/10.1080/10408398.2017.1384715

Briet A, Helsens N, Delannoy S et al (2018) NDM-1-producing *Vibrio parahaemolyticus* isolated from imported seafood. J Antimicrob Chemother 73:2578–2579. https://doi.org/10.1093/jac/dky200

Bross MH, Soch K, Morales R, Mitchell RB (2007) *Vibrio vulnificus* infection: diagnosis and treatment. AFP 76:539–544

Brown MH, Paulsen IT, Skurray RA (1999) The multidrug efflux protein NorM is a prototype of a new family of transporters. Mol Microbiol 31:394–395. https://doi.org/10.1046/j.1365-2958.1999.01162.x

Bruns MM, Kakarla P, Floyd JT et al (2017) Modulation of the multidrug efflux pump EmrD-3 from *Vibrio cholerae* by *Allium sativum* extract and the bioactive agent allyl sulfide plus synergistic enhancement of antimicrobial susceptibility by *A. sativum* extract. Arch Microbiol 199:1103–1112. https://doi.org/10.1007/s00203-017-1378-x

Burse A, Weingart H, Ullrich MS (2004) NorM, an *Erwinia amylovora* multidrug efflux pump involved in in vitro competition with other epiphytic bacteria. Appl Environ Microbiol 70:693–703. https://doi.org/10.1128/AEM.70.2.693-703.2004

Castellano S, Claxton DP, Ficici E et al (2021) Conserved binding site in the N-lobe of prokaryotic MATE transporters suggests a role for Na^+ in ion-coupled drug efflux. J Biol Chem 296:100262. https://doi.org/10.1016/j.jbc.2021.100262

CDC (2023) General Information, Cholera. https://www.cdc.gov/cholera/general/index.html. Accessed 13 Aug 2023

Ceccarelli D, Salvia AM, Sami J, Cappuccinelli P, Colombo MM (2006) New cluster of plasmid-located class 1 integrons in *Vibrio cholerae* O1 and a *dfrA15* cassette-containing integron in *Vibrio parahaemolyticus* isolated in Angola. Antimicrob Agents Chemother 50(7):2493–2499

Ceccarelli D, Chen A, Hasan NA et al (2015) Non-O1/Non-O139 *Vibrio cholerae* carrying multiple virulence factors and *V. cholerae* O1 in the Chesapeake Bay Maryland. Appl Environ Microbiol 81:1909–1918. https://doi.org/10.1128/AEM.03540-14

Chakraborty S, Nair GB, Shinoda S (1997) Pathogenic vibrios in the natural aquatic environment. Rev Environ Health 12:63–80. https://doi.org/10.1515/REVEH.1997.12.2.63

Chang G (2003) Structure of MsbA from *Vibrio cholera*: a multidrug resistance ABC transporter homolog in a closed conformation. J Mol Biol 330:419–430. https://doi.org/10.1016/s0022-2836(03)00587-4

Changsen C, Likhitrattanapisal S, Lunha K et al (2023) Incidence, genetic diversity, and antimicrobial resistance profiles of *Vibrio parahaemolyticus* in seafood in Bangkok and eastern Thailand. PeerJ 11:e15283. https://doi.org/10.7717/peerj.15283

Chatterjee P, Kanungo S, Bhattacharya SK, Dutta S (2020) Mapping cholera outbreaks and antibiotic resistant *Vibrio cholerae* in India: an assessment of existing data and a scoping review of the literature. Vaccine 38:A93–A104. https://doi.org/10.1016/j.vaccine.2019.12.003

Chen S, Wang H, Katzianer DS et al (2013) LysR family activator-regulated major facilitator superfamily transporters are involved in *Vibrio cholerae* antimicrobial compound resistance and intestinal colonisation. Int J Antimicrob Agents 41:188–192. https://doi.org/10.1016/j.ijantimicag.2012.10.008

Chen Y-T, Tang H-J, Chao C-M, Lai C-C (2015) Clinical manifestations of non-O1 *Vibrio cholerae* infections. PLoS ONE 10:e0116904. https://doi.org/10.1371/journal.pone.0116904

Chen X, Li Y, Yao W et al (2020) A new emerging serotype of *Vibrio parahaemolyticus* in China is rapidly becoming the main epidemic strain. Clin Microbiol Infect 26:644.e1-644.e7. https://doi.org/10.1016/j.cmi.2019.09.024

Chowdhury NR, Stine OC, Morris JG, Nair GB (2004) Assessment of evolution of pandemic *Vibrio parahaemolyticus* by multilocus sequence typing. J Clin Microbiol 42:1280–1282. https://doi.org/10.1128/jcm.42.3.1280-1282.2004

Claxton DP, Jagessar KL, Mchaourab HS (2021) Principles of alternating access in multidrug and toxin extrusion (MATE) transporters. J Mol Biol 433:166959. https://doi.org/10.1016/j.jmb.2021.166959

Colmer JA, Fralick JA, Hamood AN (1998) Isolation and characterization of a putative multidrug resistance pump from *Vibrio cholerae*. Mol Microbiol 27:63–72. https://doi.org/10.1046/j.1365-2958.1998.00657.x

Colwell RR (2000) Viable but nonculturable bacteria: a survival strategy. J Infect Chemother 6:121–125. https://doi.org/10.1007/PL00012151

Colwell RR, Kaper J, Joseph SW (1977) *Vibrio cholerae*, *Vibrio parahaemolyticus*, and other vibrios: occurrence and distribution in Chesapeake Bay. Science 198:394–396

Costa WF, Giambiagi-deMarval M, Laport MS (2021) Antibiotic and heavy metal susceptibility of non-cholera *Vibrio* isolated from Marine Sponges and Sea Urchins: could they pose a potential risk to public health? Antibiotics (Basel) 10:1561. https://doi.org/10.3390/antibiotics10121561

Coutinho FH, Tschoeke DA, Clementino MM et al (2019) Genomic basis of antibiotic resistance in *Vibrio parahaemolyticus* strain JPA1. Mem Inst Oswaldo Cruz 114:e190053. https://doi.org/10.1590/0074-02760190053

Cutugno L, Mc Cafferty J, Pané-Farré J et al (2020) *rpoB* mutations conferring rifampicin-resistance affect growth, stress response and motility in *Vibrio vulnificus*. Microbiology (Reading) 166:1160–1170. https://doi.org/10.1099/mic.0.000991

da Silva LV, Ossai S, Chigbu P, Parveen S (2021) Antimicrobial and genetic profiles of *Vibrio vulnificus* and *Vibrio parahaemolyticus* isolated From the Maryland Coastal Bays. United States Front Microbiol 12:676249. https://doi.org/10.3389/fmicb.2021.676249

Dahanayake PS, Hossain S, Wickramanayake MVKS et al (2020) Manila clam (*Ruditapes philippinarum*) marketed in Korea as a source of vibrios harbouring virulence and β-lactam resistance genes. Lett Appl Microbiol 71:46–53. https://doi.org/10.1111/lam.13229

Dalsgaard A, Forslund A, Petersen A et al (2000) Class 1 integron-borne, multiple-antibiotic resistance encoded by a 150-kilobase conjugative plasmid in epidemic *Vibrio cholerae* O1 strains isolated in Guinea-Bissau. J Clin Microbiol 38:3774–3779. https://doi.org/10.1128/JCM.38.10.3774-3779.2000

Das B, Verma J, Kumar P et al (2020) Antibiotic resistance in *Vibrio cholerae*: understanding the ecology of resistance genes and mechanisms. Vaccine 38(Suppl 1):A83–A92. https://doi.org/10.1016/j.vaccine.2019.06.031

Dawan J, Li Y, Lu F et al (2022) Role of efflux pump-mediated antibiotic resistance in quorum sensing-regulated biofilm formation by *Salmonella typhimurium*. Pathogens 11:147. https://doi.org/10.3390/pathogens11020147

De Jesus M, Jin J, Guffanti AA, Krulwich TA (2005) Importance of the GP dipeptide of the antiporter motif and other

membrane-embedded proline and glycine residues in tetracycline efflux protein Tet(L). Biochemistry 44:12896–12904. https://doi.org/10.1021/bi050762c

Deen J, Mengel MA, Clemens JD (2020) Epidemiology of cholera. Vaccine 38:A31–A40. https://doi.org/10.1016/j.vaccine.2019.07.078

Deepanjali A, Kumar HS, Karunasagar I (2005) Seasonal variation in abundance of total and pathogenic *Vibrio parahaemolyticus* bacteria in oysters along the southwest coast of India. Appl Environ Microbiol 71:3575–3580. https://doi.org/10.1128/AEM.71.7.3575-3580.2005

Delmar JA, Su CC, Yu EW (2014) Bacterial multidrug efflux transporters. Annu Rev Biophys 43:93–117. https://doi.org/10.1146/annurev-biophys-051013-022855

Dengo-Baloi LC, Semá-Baltazar CA, Manhique LV et al (2017) Antibiotics resistance in El Tor *Vibrio cholerae* 01 isolated during cholera outbreaks in Mozambique from 2012 to 2015. PLoS ONE 12:e0181496. https://doi.org/10.1371/journal.pone.0181496

DePaola A, Nordstrom JL, Bowers JC et al (2003) Seasonal abundance of total and pathogenic *Vibrio parahaemolyticus* in Alabama oysters. Appl Environ Microbiol 69:1521–1526

Deshayes S, Daurel C, Cattoir V et al (2015) Non-O1, non-O139 *Vibrio cholerae* bacteraemia: case report and literature review. Springerplus 4:575. https://doi.org/10.1186/s40064-015-1346-3

Destoumieux-Garzón D, Duperthuy M, Vanhove AS et al (2014) Resistance to antimicrobial peptides in vibrios. Antibiotics (basel) 3:540–563. https://doi.org/10.3390/antibiotics3040540

Dhakne P, Pillai M, Mishra S et al (2023) Refinement of safety and efficacy of anti-cancer chemotherapeutics by tailoring their site-specific intracellular bioavailability through transporter modulation. Biochim Biophys Acta 1878:188906. https://doi.org/10.1016/j.bbcan.2023.188906

Du D, Wang-Kan X, Neuberger A et al (2018) Multidrug efflux pumps: structure, function and regulation. Nat Rev Microbiol 16:523–539. https://doi.org/10.1038/s41579-018-0048-6

Dubon JM, Palmer CJ, Ager AL et al (1997) Emergence of multiple drug-resistant *Vibrio cholerae* O1 in San Pedro Sula. Honduras Lancet 349:924. https://doi.org/10.1016/s0140-6736(05)62699-2

Dutta D, Chowdhury G, Pazhani GP et al (2013) *Vibrio cholerae* non-O1, non-o139 serogroups and cholera-like diarrhea, Kolkata, India. Emerg Infect Dis 19:464–467. https://doi.org/10.3201/eid1903.121156

Dutta D, Kaushik A, Kumar D, Bag S (2021) Foodborne pathogenic vibrios: antimicrobial resistance. Front Microbiol 12:638331. https://doi.org/10.3389/fmicb.2021.638331

ECDC (2023) Cholera worldwide overview. https://www.ecdc.europa.eu/en/all-topics-z/cholera/surveillance-and-disease-data/cholera-monthly. Accessed 14 Aug 2023

Elmahdi S, DaSilva LV, Parveen S (2016) Antibiotic resistance of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in various countries: a review. Food Microbiol 57:128–134. https://doi.org/10.1016/j.fm.2016.02.008

Evans K, Passador L, Srikumar R et al (1998) Influence of the MexAB-OprM multidrug efflux system on quorum sensing in *Pseudomonas aeruginosa*. J Bacteriol 180:5443–5447

Faruque SM, Kamruzzaman M, Meraj IM et al (2003a) Pathogenic potential of environmental *Vibrio cholerae* strains carrying genetic variants of the toxin-coregulated pilus pathogenicity Island. Infect Immun 71:1020–1025. https://doi.org/10.1128/IAI.71.2.1020-1025.2003

Faruque SM, Sack DA, Sack RB et al (2003b) Emergence and evolution of *Vibrio cholerae* O139. Proc Natl Acad Sci U S A 100:1304–1309. https://doi.org/10.1073/pnas.0337468100

Fernández-Vélez I, Bidegain G, Ben-Horin T (2023) Predicting the growth of *Vibrio parahaemolyticus* in oysters under varying ambient temperature. Microorganisms 11:1169. https://doi.org/10.3390/microorganisms11051169

Ghenem L, Elhadi N, Alzahrani F, Nishibuchi M (2017) *Vibrio parahaemolyticus*: a review on distribution, pathogenesis, virulence determinants and epidemiology. Saudi J Med Med Sci 5:93–103. https://doi.org/10.4103/sjmms.sjmms_30_17

Ginn SL, Brown MH, Skurray RA (2000) The TetA (K) tetracycline/H^+ antiporter from *Staphylococcus aureus*: mutagenesis and functional analysis of motif C. J Bacteriol 182:1492–1498

Gladkikh AS, Feranchuk SI, Ponomareva AS et al (2020) Antibiotic resistance in *Vibrio cholerae* El Tor strains isolated during cholera complications in Siberia and the Far East of Russia. Infect Genet Evol 78:104096. https://doi.org/10.1016/j.meegid.2019.104096

Glass RI, Huq I, Alim AR, Yunus M (1980) Emergence of multiply antibiotic-resistant *Vibrio cholerae* in Bangladesh. J Infect Dis 142:939–942. https://doi.org/10.1093/infdis/142.6.939

Griffith JK, Baker ME, Rouch DA et al (1992) Membrane transport proteins: implications of sequence comparisons. Curr Opin Cell Biol 4:684–695. https://doi.org/10.1016/0955-0674(92)90090-y

Griffith JK, Cuellar DH, Fordyce CA et al (1994) Structure and function of the class C tetracycline/H^+ antiporter: three independent groups of phenotypes are conferred by TetA (C). Mol Membr Biol 11:271–277. https://doi.org/10.3109/09687689409160437

Grudlewska-Buda K, Bauza-Kaszewska J, Wiktorczyk-Kapischke N et al (2023) Antibiotic resistance in selected emerging bacterial foodborne pathogens-an issue of concern? Antibiotics (basel) 12:880. https://doi.org/10.3390/antibiotics12050880

Guillaume Y, Ternier R, Vissieres K et al (2018) Responding to cholera in Haiti: implications for the national plan to eliminate cholera by 2022. J Infect Dis 218:S167–S170. https://doi.org/10.1093/infdis/jiy491

Gulig PA, Bourdage KL, Starks AM (2005) Molecular pathogenesis of *Vibrio vulnificus*. J Microbiol 43:118–131

Hammer BK, Bassler BL (2003) Quorum sensing controls biofilm formation in *Vibrio cholerae*. Mol Microbiol 50:101–104

Han F, Walker RD, Janes ME et al (2007) Antimicrobial susceptibilities of *Vibrio parahaemolyticus* and *Vibrio vulnificus* isolates from Louisiana Gulf and retail raw Oysters. Appl Environ Microbiol 73:7096–7098. https://doi.org/10.1128/AEM.01116-07

Hao Y, Wang Y, Bi Z et al (2015) A case of non-O1/non-O139 *Vibrio cholerae* septicemia and meningitis in a neonate. Int J Infect Dis 35:117–119. https://doi.org/10.1016/j.ijid.2015.05.004

Harris JB, LaRocque RC, Qadri F et al (2012) Cholera. Lancet 379:2466–2476. https://doi.org/10.1016/S0140-6736(12)60436-X

Hassan KA, Galea M, Wu J et al (2006) Functional effects of intramembranous proline substitutions in the staphylococcal multidrug transporter QacA. FEMS Microbiol Lett 263:76–85

Hassan KA, Maher C, Elbourne LD et al (2021) Increasing the PACE of characterising novel transporters by functional genomics. Curr Opin Microbiol 64:1–8. https://doi.org/10.1016/j.mib.2021.08.005

He G-X, Kuroda T, Mima T et al (2004) An H^+-coupled multidrug efflux pump, PmpM, a member of the MATE family of transporters, from *Pseudomonas aeruginosa*. J Bacteriol 186:262–265. https://doi.org/10.1128/JB.186.1.262-265.2004

He X, Szewczyk P, Karyakin A et al (2010) Structure of a cation-bound multidrug and toxic compound extrusion transporter. Nature 467:991–994. https://doi.org/10.1038/nature09408

Hedges RW, Jacob AE (1975) A 98 megadalton R factor of compatibility group C in a *Vibrio cholerae* El Tor isolate from Southern U.S.S.R. Microbiology 89:383–386. https://doi.org/10.1099/00221287-89-2-383

Heidelberg JF, Eisen JA, Nelson WC et al (2000) DNA sequence of both chromosomes of the cholera pathogen *Vibrio cholerae*. Nature 406:477–483. https://doi.org/10.1038/35020000

Henderson PJ (1990) The homologous glucose transport proteins of prokaryotes and eukaryotes. Res Microbiol 141:316–328. https://doi.org/10.1016/0923-2508(90)90005-b

Henderson PJ (1993) The 12-transmembrane helix transporters. Curr Opin Cell Biol 5:708–721. https://doi.org/10.1016/0955-0674(93)90144-f

Heng S-P, Letchumanan V, Deng C-Y et al (2017) *Vibrio vulnificus*: an environmental and clinical burden. Front Microbiol. https://doi.org/10.3389/fmicb.2017.00997

Higgins DA, Pomianek ME, Kraml CM et al (2007) The major *Vibrio cholerae* autoinducer and its role in virulence factor production. Nature 450:883–886

Hirota D, Sachdev A, Thrupp L et al (2010) Traumatic arm wound infected with *Vibrio cholerae* in a non-immunocompromised Host. https://www.hmpgloballearningnetwork.com/site/wounds/case-report-and-brief-review/traumatic-arm-wound-infected-vibrio-cholerae-non. Accessed 9 Oct 2023

Hong To TT, Yanagawa H, Khanh Thuan N et al (2020) Prevalence of *Vibrio parahaemolyticus* causing acute hepatopancreatic necrosis disease of shrimp in shrimp, Molluscan shellfish and water samples in the Mekong Delta, Vietnam. Biology 9:312. https://doi.org/10.3390/biology9100312

HongYou C, LiHong T, Min C et al (2014) Investigation of diversity of *Vibrio parahaemolyticus* in molluscs. Dis Surveill 29:522–527

Horseman MA, Surani S (2011) A comprehensive review of *Vibrio vulnificus*: an important cause of severe sepsis and skin and soft-tissue infection. Int J Infect Dis 15:e157–e166. https://doi.org/10.1016/j.ijid.2010.11.003

Hossain S, Wickramanayake MVKS, Dahanayake PS, Heo GJ (2020) Occurrence of virulence and extended-spectrum β-lactamase determinants in *Vibrio* spp. isolated from marketed hard-shelled mussel (*Mytilus coruscus*). Microb Drug Resist 26:391–401. https://doi.org/10.1089/mdr.2019.0131

Hou Z, Gangjee A, Matherly LH (2022) The evolving biology of the proton-coupled folate transporter: new insights into regulation, structure, and mechanism. FASEB J 36:e22164. https://doi.org/10.1096/fj.202101704R

Hounmanou YMG, Engberg J, Bjerre KD, et al (2023) Correlation of high seawater temperature with Vibrio and Shewanella infections, Denmark, 2010–2018 - Volume 29, Number 3—March 2023 - Emerg Infect Dis J CDC. https://doi.org/10.3201/eid2903.221568

Huda MN, Chen J, Morita Y et al (2003) Gene cloning and characterization of VcrM, a Na^+-coupled multidrug efflux pump, from *Vibrio cholerae* non-O1. Microbiol Immunol 47:419–427. https://doi.org/10.1111/j.1348-0421.2003.tb03379.x

Huq A, Colwell RR (1996) A microbiological paradox: viable but non-culturable bacteria with special reference to *Vibrio cholerae*. J Food Prot 59:96–101. https://doi.org/10.4315/0362-028X-59.1.96

Hvorup RN, Winnen B, Chang AB et al (2003) The multidrug/oligosaccharidyl-lipid/polysaccharide (MOP) exporter superfamily. Eur J Biochem 270:799–813

Ichinose Y, Ehara M, Watanabe S et al (1986) The characterization of *Vibrio cholerae* isolated in Kenya in 1983. J Trop Med Hyg 89:269–276

Ingelbeen B, Hendrickx D, Miwanda B et al (2019) Recurrent cholera outbreaks, Democratic Republic of the Congo, 2008–2017. Emerg Infect Dis 25:856

Jack DL, Yang NM, Saier MH Jr (2001) The drug/metabolite transporter superfamily. Eur J Biochem 268:3620–3639

Jain M, Kumar P, Goel AK (2016) Emergence of tetracycline resistant *Vibrio cholerae* O1 Biotype El Tor serotype Ogawa with classical *ctxB* gene from a cholera outbreak in Odisha, Eastern India. J Pathog 2016:1695410. https://doi.org/10.1155/2016/1695410

Jiang D, Zhao Y, Wang X et al (2013) Structure of the YajR transporter suggests a transport mechanism based on the conserved motif A. Proc Natl Acad Sci USA 110:14664–14669. https://doi.org/10.1073/pnas.1308127110

Jin J, Krulwich TA (2002) Site-directed mutagenesis studies of selected motif and charged residues and of cysteines of the multifunctional tetracycline efflux protein Tet(L). J Bacteriol 184:1796–1800. https://doi.org/10.1128/jb.184.6.1796-1800.2002

Jin Y, Nair A, van Veen HW (2014) Multidrug transport protein NorM from *Vibrio cholerae* simultaneously couples to sodium- and proton-motive force. J Biol Chem 289:14624–14632. https://doi.org/10.1074/jbc.M113.546770

Jones MK, Oliver JD (2009) *Vibrio vulnificus*: disease and pathogenesis. Infect Immun 77:1723–1733. https://doi.org/10.1128/IAI.01046-08

Kakarla P, Floyd J, Mukherjee M et al (2017) Inhibition of the multidrug efflux pump LmrS from *Staphylococcus aureus* by cumin spice *Cuminum cyminum*. Arch Microbiol 199:465–474. https://doi.org/10.1007/s00203-016-1314-5

Kaper JB, Morris JG, Levine MM (1995) Cholera. Clin Microbiol Rev 8:48–86

Kathleen MM, Samuel L, Felecia C, et al (2016) Antibiotic resistance of diverse bacteria from aquaculture in Borneo. Int J Microbiol 2016:2164761. https://www.hindawi.com/journals/ijmicro/2016/2164761/.

Kazama H, Hamashima H, Sasatsu M, Arai T (1999) Characterization of the antiseptic-resistance gene *qacEΔ1* isolated from clinical and environmental isolates of *Vibrio parahaemolyticus* and *Vibrio cholerae* non-O1. FEMS Microbiol Lett 174(2):379–384

Kitaoka M, Miyata ST, Unterweger D, Pukatzki S (2011) Antibiotic resistance mechanisms of *Vibrio cholerae*. J Med Microbiol 60:397–407. https://doi.org/10.1099/jmm.0.023051-0

Konishi S, Iwaki S, Kimura-Someya T, Yamaguchi A (1999) Cysteine-scanning mutagenesis around transmembrane segment VI of Tn10-encoded metal-tetracycline/H(+) antiporter. FEBS Lett 461:315–318. https://doi.org/10.1016/s0014-5793(99)01490-8

Kumar S, He G, Kakarla P et al (2016a) Bacterial multidrug efflux pumps of the major facilitator superfamily as targets for modulation. Infect Disord Drug Targets 16:28–43. https://doi.org/10.2174/1871526516666160407113848

Kumar S, Ranjana K, Sanford LM et al (2016b) Structural and functional roles of two evolutionarily conserved amino acid sequence motifs within solute transporters of the major facilitator superfamily. Trends Cell Mol Biol 11:41–53

Kumar S, Lindquist IE, Sundararajan A et al (2013a) Genome sequence of non-O1 *Vibrio cholerae* PS15. Genome Announc. https://doi.org/10.1128/genomeA.00227-12

Kumar S, Mukherjee MM, Varela MF (2013b) Modulation of bacterial multidrug resistance efflux pumps of the major facilitator superfamily. Int J Bacteriol 2013:204141. https://doi.org/10.1155/2013/204141

Kumar S, Lekshmi M, Parvathi A et al (2017) Antibiotic resistance in seafood-borne pathogens. In: Foodborne pathogens and antibiotic resistance. Wiley, pp 397–415. https://doi.org/10.1002/9781119139188.ch17

Kumar S, Lekshmi M, Parvathi A et al (2020) Functional and structural roles of the major facilitator superfamily bacterial multidrug efflux pumps. Microorganisms 8:266. https://doi.org/10.3390/microorganisms8020266

Kunkle DE, Bina XR, Bina JE (2017) The *Vibrio cholerae* VexGH RND efflux system maintains cellular homeostasis by effluxing Vibriobactin. Mbio. https://doi.org/10.1128/mBio.00126-17

Kuroda T, Tsuchiya T (2009) Multidrug efflux transporters in the MATE family. Biochim Biophys Acta 1794(5):763–768. https://doi.org/10.1016/j.bbapap.2008.11.012

Kusakizako T, Miyauchi H, Ishitani R, Nureki O (2020) Structural biology of the multidrug and toxic compound extrusion superfamily transporters. Biochim Et Biophys Acta BBA Biomembr 1862:183154. https://doi.org/10.1016/j.bbamem.2019.183154

Kuwahara S, Goto S, Kimura M, Abe H (1967) Drug-sensitivity of El Tor vibrio strains isolated in the Philippines in 1964 and 1965. Bull World Health Organ 37:763–771

Lee S, Yeom J-H, Seo S et al (2015) Functional analysis of *Vibrio vulnificus* RND efflux pumps homologous to *Vibrio cholerae* VexAB and VexCD, and to *Escherichia coli* AcrAB. J Microbiol 53:256–261. https://doi.org/10.1007/s12275-015-5037-0

Lee L-H, Ab Mutalib N-S, Law JW-F et al (2018) Discovery on antibiotic resistance patterns of *Vibrio parahaemolyticus* in selangor reveals carbapenemase producing *Vibrio parahaemolyticus* in marine and freshwater fish. Front Microbiol 9:2513. https://doi.org/10.3389/fmicb.2018.02513

Lei T, Zhang J, Jiang F et al (2019) First detection of the plasmid-mediated colistin resistance gene *mcr-1* in virulent *Vibrio parahaemolyticus*. Int J Food Microbiol 308:108290. https://doi.org/10.1016/j.ijfoodmicro.2019.108290

Lei T, Jiang F, He M et al (2020) Prevalence, virulence, antimicrobial resistance, and molecular characterization of fluoroquinolone resistance of *Vibrio parahaemolyticus* from different types of food samples in China. Int J Food Microbiol 317:108461. https://doi.org/10.1016/j.ijfoodmicro.2019.108461

Lekshmi M, Ammini P, Kumar S, Varela MF (2017) The food production environment and the development of antimicrobial resistance in human pathogens of animal origin. Microorganisms. https://doi.org/10.3390/microorganisms5010011

Letchumanan V, Yin W-F, Lee L-H, Chan K-G (2015) Prevalence and antimicrobial susceptibility of *Vibrio parahaemolyticus* isolated from retail shrimps in Malaysia. Front Microbiol 6:33. https://doi.org/10.3389/fmicb.2015.00033

Li G, Wang M-Y (2020) The role of *Vibrio vulnificus* virulence factors and regulators in its infection-induced sepsis. Folia Microbiol (praha) 65:265–274. https://doi.org/10.1007/s12223-019-00763-7

Lipp EK, Huq A, Colwell RR (2002) Effects of global climate on infectious disease: the cholera model. Clin Microbiol Rev 15:757–770. https://doi.org/10.1128/CMR.15.4.757-770.2002

Liu P, Zhang X, Wang Q et al (2022) Biological and transcriptional studies reveal VmeL is involved in motility, biofilm formation and virulence in *Vibrio parahaemolyticus*. Front Microbiol 13:976334

Lloyd NA, Nazaret S, Barkay T (2019) Genome-facilitated discovery of RND efflux pump-mediated resistance to cephalosporins in *Vibrio* spp. isolated from the mummichog fish gut. J Glob Antimicrob Resist 19:294–300

Lo C-C, Lin P-T, Chiang-Ni C et al (2017) Contribution of efflux systems to the detergent resistance, cytotoxicity, and biofilm formation of *Vibrio vulnificus*. Gene Rep 9:115–122. https://doi.org/10.1016/j.genrep.2017.09.004

Lomovskaya O, Lewis K (1992) Emr, an *Escherichia coli* locus for multidrug resistance. Proc Natl Acad Sci USA 89:8938–8942. https://doi.org/10.1073/pnas.89.19.8938

Loo KY, Letchumanan V, Law JWF et al (2020) Incidence of antibiotic resistance in *Vibrio* spp. Rev Aquac 12:2590–2608. https://doi.org/10.1111/raq.12460

Lu M, Symersky J, Radchenko M et al (2013) Structures of a Na^+-coupled, substrate-bound MATE multidrug transporter. Proc Natl Acad Sci USA 110:2099–2104. https://doi.org/10.1073/pnas.1219901110

Lu W-J, Lin H-J, Janganan TK et al (2018) ATP-binding cassette transporter VcaM from *Vibrio cholerae* is dependent on the outer membrane factor family for its function. Int J Mol Sci 19:E1000. https://doi.org/10.3390/ijms19041000

Luo J, Parsons SM (2010) Conformational propensities of peptides mimicking transmembrane Helix 5 and Motif C in wild-type and mutant vesicular acetylcholine transporters. ACS Chem Neurosci 1:381–390. https://doi.org/10.1021/cn900033s

Maddocks SE, Oyston PCF (2008) Structure and function of the LysR-type transcriptional regulator (LTTR) family proteins. Microbiology (reading) 154:3609–3623. https://doi.org/10.1099/mic.0.2008/022772-0

Maiden MC, Davis EO, Baldwin SA et al (1987) Mammalian and bacterial sugar transport proteins are homologous. Nature 325:641–643

Mandal A, Sengupta A, Kumar A et al (2017) Molecular epidemiology of extended-spectrum β-lactamase–producing *Escherichia coli* pathotypes in diarrheal children from low socioeconomic status communities in Bihar, India: emergence of the CTX-M Type. Infect Dis (auckl) 10:1178633617739018. https://doi.org/10.1177/1178633617739018

Manjusha S, Sarita GB (2011) Plasmid associated antibiotic resistance in Vibrios isolated from coastal waters of Kerala. Int Food Res J 18:1171–1181

Marinello S, Marini G, Parisi G et al (2017) *Vibrio cholerae* non-O1, non-O139 bacteraemia associated with pneumonia, Italy 2016. Infection 45:237–240. https://doi.org/10.1007/s15010-016-0961-4

Martínez JL (2008) Antibiotics and antibiotic resistance genes in natural environments. Science 321:365–367

Maseda H, Sawada I, Saito K et al (2004) Enhancement of the *mexAB-oprM* efflux pump expression by a quorum-sensing autoinducer and its cancellation by a regulator, MexT, of the *mexEF-oprN* efflux pump operon in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 48:1320–1328. https://doi.org/10.1128/aac.48.4.1320-1328.2004

Masureel M, Martens C, Stein RA et al (2014) Protonation drives the conformational switch in the multidrug transporter LmrP. Nat Chem Biol 10:149–155. https://doi.org/10.1038/nchembio.1408

Matsumoto C, Okuda J, Ishibashi M et al (2000) Pandemic spread of an O3:K6 clone of *Vibrio parahaemolyticus* and emergence of related strains evidenced by arbitrarily primed PCR and toxRS sequence analyses. J Clin Microbiol 38:578–585

Matsuo T, Hayashi K, Morita Y et al (2007) VmeAB, an RND-type multidrug efflux transporter in *Vibrio parahaemolyticus*. Microbiology 153:4129–4137. https://doi.org/10.1099/mic.0.2007/009597-0

Matsuo T, Nakamura K, Kodama T et al (2013) Characterization of all RND-type multidrug efflux transporters in *Vibrio parahaemolyticus*. MicrobiologyOpen 2:725–742. https://doi.org/10.1002/mbo3.100

McMurry L, Petrucci RE Jr, Levy BS (1980) Active efflux of tetracycline encoded by four genetically different tetracycline resistance determinants in *Escherichia coli*. Proc Natl Acad Sci 77:3974–3977

Meng X, Huang D, Zhou Q et al (2023) The influence of outer membrane protein on ampicillin resistance of *Vibrio parahaemolyticus*. Can J Infect Dis Med Microbiol 2023:8079091. https://doi.org/10.1155/2023/8079091

Mevada V, Patel R, Dudhagara P et al (2023) Whole genome sequencing and pan-genomic analysis of multidrug-resistant *Vibrio cholerae* VC01 isolated from a clinical sample. Microorganisms 11:2030. https://doi.org/10.3390/microorganisms11082030

Miyamae S, Ueda O, Yoshimura F, Hwang J, Tanaka Y, Nikaido H (2001) A MATE family multidrug efflux transporter pumps out fluoroquinolones in *Bacteroides thetaiotaomicron*. Antimicrob

Agents Chemother 45(12):3341–3346. https://doi.org/10.1128/aac.45.12.3341-3346.2001
Mohanty P, Patel A, Bhardwaj AK (2012) Role of H- and D- MATE-type transporters from multidrug resistant clinical isolates of *Vibrio fluvialis* in conferring fluoroquinolone resistance. PLoS ONE 7:e35752. https://doi.org/10.1371/journal.pone.0035752
Mohanty P, Shah A, Bhardwaj AK (2021) Functional insights of two MATE transporters from *Vibrio fluvialis*. bioRxiv 49:949
Mok JS, Ryu A, Kwon JY et al (2019) Abundance, antimicrobial resistance, and virulence of pathogenic Vibrio strains from molluscan shellfish farms along the Korean coast. Mar Pollut Bull 149:110559. https://doi.org/10.1016/j.marpolbul.2019.110559
Molina-Aja A, García-Gasca A, Abreu-Grobois A et al (2002) Plasmid profiling and antibiotic resistance of Vibrio strains isolated from cultured penaeid shrimp. FEMS Microbiol Lett 213:7–12. https://doi.org/10.1016/S0378-1097(02)00791-7
Moorthy S, Watnick PI (2005) Identification of novel stage-specific genetic requirements through whole genome transcription profiling of *Vibrio cholerae* biofilm development. Mol Microbiol 57:1623–1635. https://doi.org/10.1111/j.1365-2958.2005.04797.x
Morita Y, Kataoka A, Shiota S et al (2000) NorM of vibrio parahaemolyticus is an Na(+)-driven multidrug efflux pump. J Bacteriol 182:6694–6697. https://doi.org/10.1128/JB.182.23.6694-6697.2000
Morita D, Takahashi E, Morita M et al (2020) Genomic characterization of antibiotic resistance-encoding genes in clinical isolates of *Vibrio cholerae* non-O1/non-O139 strains from Kolkata, India: generation of novel types of genomic islands containing plural antibiotic resistance genes. Microbiol Immunol 64:435–444. https://doi.org/10.1111/1348-0421.12790
Morita Y, Li X-Z (2016) Antimicrobial resistance and drug efflux pumps in *Vibrio* and *Legionella*. In: Li XZ, Elkins C, Zgurskaya H (eds) Efflux-mediated antimicrobial resistance in bacteria. Adis, Cham, p 307–328
Morris JG (2003) Cholera and other types of vibriosis: a story of human pandemics and oysters on the half shell. Clin Infect Dis 37:272–280. https://doi.org/10.1086/375600
Morris RD (2007) The blue death : disease, disaster, and the water we drink, 1st edn. HarperCollins, New York
Motes ML, DePaola A, Cook DW et al (1998) Influence of water temperature and salinity on *Vibrio vulnificus* in Northern Gulf and Atlantic Coast oysters (*Crassostrea virginica*). Appl Environ Microbiol 64:1459–1465. https://doi.org/10.1128/AEM.64.4.1459-1465.1998
Mukherjee M, Kakarla P, Kumar S et al (2014) Comparative genome analysis of non-toxigenic non-O1 versus toxigenic O1 *Vibrio cholerae*. Genom Discov 2:1–15. https://doi.org/10.7243/2052-7993-2-1
Mukhopadhyay AK, Takeda Y, Balakrish Nair G (2014) Cholera outbreaks in the El Tor Biotype era and the impact of the new El Tor variants. In: Nair GB, Takeda Y (eds) Cholera outbreaks. Springer, Berlin, pp 17–47
Nair GB, Ramamurthy T, Bhattacharya SK et al (1994) Spread of *Vibrio cholerae* O139 Bengal in India. J Infect Dis 169:1029–1034. https://doi.org/10.1093/infdis/169.5.1029
Nakayama T, Yamaguchi T, Jinnai M et al (2023) ESBL-producing *Vibrio vulnificus* and *V. alginolyticus* harbour a plasmid encoding ISE*c*9 upstream of $bla_{CTX\text{-}M\text{-}55}$ and *qnrS2* isolated from imported seafood. Arch Microbiol 205:241. https://doi.org/10.1007/s00203-023-03569-x
Ndraha N, Hsiao H-I (2019) The risk assessment of *Vibrio parahaemolyticus* in raw oysters in Taiwan under the seasonal variations, time horizons, and climate scenarios. Food Control 102:188–196. https://doi.org/10.1016/j.foodcont.2019.03.020
Nelson ML, Levy SB (2011) The history of the tetracyclines. Ann N Y Acad Sci 1241:17–32. https://doi.org/10.1111/j.1749-6632.2011.06354.x
Neogi SB, Chowdhury N, Awasthi SP et al (2019) Novel cholera toxin variant and ToxT regulon in environmental *Vibrio mimicus* isolates: potential resources for the evolution of *Vibrio cholerae* hybrid strains. Appl Environ Microbiol 85:e01977-e2018. https://doi.org/10.1128/AEM.01977-18
Nikaido H (1994) Prevention of drug access to bacterial targets: permeability barriers and active efflux. Science 264:382–388. https://doi.org/10.1126/science.8153625
Nikaido H (1998) Antibiotic resistance caused by gram-negative multidrug efflux pumps. Clin Infect Dis 27:S32–S41. https://doi.org/10.1086/514920
Nikaido H (2011) Structure and mechanism of RND-type multidrug efflux pumps. Adv Enzymol Relat Areas Mol Biol 77:1–60. https://doi.org/10.1002/9780470920541.ch1
Nishibuchi M, Ishibashi M, Takeda Y, Kaper JB (1985) Detection of the thermostable direct hemolysin gene and related DNA sequences in *Vibrio parahaemolyticus* and other vibrio species by the DNA colony hybridization test. Infect Immun 49:481–486. https://doi.org/10.1128/IAI.49.3.481-486.1985
Nishibuchi M, Taniguchi T, Misawa T et al (1989) Cloning and nucleotide sequence of the gene (*trh*) encoding the hemolysin related to the thermostable direct hemolysin of *Vibrio parahaemolyticus*. Infect Immun 57:2691–2697. https://doi.org/10.1128/IAI.57.9.2691-2697.1989
Ogawa W, Minato Y, Dodan H et al (2015) Characterization of MATE-type multidrug efflux pumps from *Klebsiella pneumoniae* MGH78578. PLoS ONE 10:e0121619. https://doi.org/10.1371/journal.pone.0121619
Oh EG, Son KT, Yu H et al (2011) Antimicrobial resistance of *Vibrio parahaemolyticus* and *Vibrio alginolyticus* strains isolated from farmed fish in Korea from 2005 through 2007. J Food Prot 74:380–386. https://doi.org/10.4315/0362-028X.JFP-10-307
Okoh AI, Igbinosa EO (2010) Antibiotic susceptibility profiles of some Vibrio strains isolated from wastewater final effluents in a rural community of the Eastern Cape Province of South Africa. BMC Microbiol 10:143. https://doi.org/10.1186/1471-2180-10-143
Okuda J, Ishibashi M, Hayakawa E et al (1997) Emergence of a unique O3:K6 clone of *Vibrio parahaemolyticus* in Calcutta, India, and isolation of strains from the same clonal group from Southeast Asian travelers arriving in Japan. J Clin Microbiol 35:3150–3155
Okura M, Osawa R, Iguchi A et al (2003) Genotypic analyses of *Vibrio parahaemolyticus* and development of a pandemic group-specific multiplex PCR assay. J Clin Microbiol 41:4676–4682
Oliver JD (2010) Recent findings on the viable but nonculturable state in pathogenic bacteria. FEMS Microbiol Rev 34:415–425. https://doi.org/10.1111/j.1574-6976.2009.00200.x
Oliver JD, Pruzzo C, Vezzulli L, Kaper JB (2012) Vibrio species. Food microbiology. John Wiley & Sons Ltd, pp 401–439
Otsuka M, Yasuda M, Morita Y et al (2005) Identification of essential amino acid residues of the NorM Na+/multidrug antiporter in *Vibrio parahaemolyticus*. J Bacteriol 187:1552–1558. https://doi.org/10.1128/JB.187.5.1552-1558.2005
Ottaviani D, Bacchiocchi I, Masini L et al (2001) Antimicrobial susceptibility of potentially pathogenic halophilic vibrios isolated from seafood. Int J Antimicrob Agents 18:135–140. https://doi.org/10.1016/S0924-8579(01)00358-2
Ottaviani D, Leoni F, Rocchegiani E et al (2011) Unusual case of necrotizing fasciitis caused by *Vibrio cholerae* O137. J Clin Microbiol 49:757–759. https://doi.org/10.1128/JCM.02257-10
Ouellette M, Gerbaud G, Courvalin P (1988) Genetic, biochemical and molecular characterization of strains of *Vibrio cholerae* multiresistant to antibiotics. Ann Inst Pasteur Microbiol 139:105–113

Oyelade AA, Adelowo OO, Fagade OE (2018) blaNDM-1-producing *Vibrio parahaemolyticus* and *V. vulnificus* isolated from recreational beaches in Lagos Nigeria. Environ Sci Pollut Res Int 25:33538–33547. https://doi.org/10.1007/s11356-018-3306-2

Pao SS, Paulsen IT, Saier MH Jr (1998) Major facilitator superfamily. Microbiol Mol Biol Rev 62:1–34

Parthasarathy S, Das SC, Kumar A et al (2021) Molecular characterization and antibiotic resistance of *Vibrio parahaemolyticus* from Indian oyster and their probable implication in food chain. World J Microbiol Biotechnol 37:145. https://doi.org/10.1007/s11274-021-03113-3

Parvathi A, Kumar HS, Karunasagar I, Karunasagar I (2004) Detection and enumeration of *Vibrio vulnificus* in oysters from two estuaries along the southwest coast of India, using molecular methods. Appl Environ Microbiol 70:6909–6913. https://doi.org/10.1128/AEM.70.11.6909-6913.2004

Pasrija R, Banerjee D, Prasad R (2007) Structure and function analysis of CaMdr1p, a major facilitator superfamily antifungal efflux transporter protein of *Candida albicans*: identification of amino acid residues critical for drug/H^+ transport. Eukaryot Cell 6:443–453. https://doi.org/10.1128/EC.00315-06

Pérez-Acosta JA, Martínez-Porchas M, Elizalde-Contreras JM et al (2018) Proteomic profiling of integral membrane proteins associated to pathogenicity in *Vibrio parahaemolyticus* strains. Microbiol Immunol 62:14–23. https://doi.org/10.1111/1348-0421.12556

Piddock LJ (2006) Multidrug-resistance efflux pumps—not just for resistance. Nat Rev Microbiol 4:629–636

Prescott LM, Datta A, Datta GC (1968) R-factors in Calcutta strains of *Vibrio cholerae* and members of the enterobacteriaceae. Bull World Health Organ 39:971–973

Rahal K, Gerbaud GR, Chabbert YA (1973) Properties of a transferable resistance factor in *Vibrio cholerae* Biotype El Tor. Ann Microbiol (Paris) 124:283–294

Rahman MM, Matsuo T, Ogawa W et al (2007) Molecular cloning and characterization of all RND-type efflux transporters in *Vibrio cholerae* non-O1. Microbiol Immunol 51:1061–1070. https://doi.org/10.1111/j.1348-0421.2007.tb04001.x

Rahmati S, Yang S, Davidson AL, Zechiedrich EL (2002) Control of the AcrAB multidrug efflux pump by quorum-sensing regulator SdiA. Mol Microbiol 43:677–685

Ramamurthy T, Garg S, Sharma R et al (1993) Emergence of novel strain of *Vibrio cholerae* with epidemic potential in southern and eastern India. Lancet 341:703–704. https://doi.org/10.1016/0140-6736(93)90480-5

Ramamurthy T, Ghosh A (2021) A re-look at cholera pandemics from early times to now in the current era of epidemiology. J Disast Res 16:110–117. https://doi.org/10.20965/jdr.2021.p0110

Ranaweera I, Shrestha U, Ranjana K et al (2015) Structural comparison of bacterial multidrug efflux pumps of the major facilitator superfamily. Trends Cell Mol Biol 10:131

Reguera G, Kolter R (2005) Virulence and the environment: a novel role for *Vibrio cholerae* toxin-coregulated pili in biofilm formation on Chitin. J Bacteriol 187:3551–3555. https://doi.org/10.1128/jb.187.10.3551-3555.2005

Reidl J, Klose KE (2002) *Vibrio cholerae* and cholera: out of the water and into the host. FEMS Microbiol Rev 26:125–139. https://doi.org/10.1111/j.1574-6976.2002.tb00605.x

Reyhanath PV, Ranjeet K (2014) Incidence of multidrug resistant *Vibrio parahaemolyticus* isolated from Ponnani, South India. Iran J Microbiol 6:60–67

Rouch DA, Cram DS, DiBerardino D et al (1990) Efflux-mediated antiseptic resistance gene qacA from *Staphylococcus aureus*: common ancestry with tetracycline- and sugar-transport proteins. Mol Microbiol 4:2051–2062. https://doi.org/10.1111/j.1365-2958.1990.tb00565.x

Rouquette-Loughlin C, Dunham SA, Kuhn M et al (2003) The NorM efflux pump of *Neisseria gonorrhoeae* and *Neisseria meningitidis* recognizes antimicrobial cationic compounds. J Bacteriol 185:1101–1106. https://doi.org/10.1128/JB.185.3.1101-1106.2003

Rubin DHF, Zingl FG, Leitner DR et al (2022) Reemergence of cholera in Haiti. N Engl J Med 387:2387–2389. https://doi.org/10.1056/NEJMc2213908

Saha GK, Ganguly NK (2021) Spread and endemicity of cholera in india: factors beyond the numbers. J Infect Dis 224:S710–S716. https://doi.org/10.1093/infdis/jiab436

Saier MH Jr, Beatty JT, Goffeau A et al (1999) The major facilitator superfamily. J Mol Microbiol Biotechnol 1:257–279

Saier MH, Reddy VS, Moreno-Hagelsieb G et al (2021) The transporter classification database (TCDB): 2021 update. Nucleic Acids Res 49:D461–D467. https://doi.org/10.1093/nar/gkaa1004

Saraswathi K, Deodhar LP (1990) A study of *V. cholerae* strains isolated in Bombay. J Postgrad Med 36:128–130

Shannon JD, Kimbrough RC (2006) Pulmonary cholera due to infection with a non-O1 *Vibrio cholerae* strain. J Clin Microbiol 44:3459–3460. https://doi.org/10.1128/jcm.02343-05

Sharma C, Thungapathra M, Ghosh A et al (1998) Molecular analysis of non-O1, non-O139 *Vibrio cholerae* associated with an unusual upsurge in the incidence of cholera-like disease in Calcutta, India. J Clin Microbiol 36:756–763. https://doi.org/10.1128/jcm.36.3.756-763.1998

Sherman I (2007) Cholera. Twelve diseases that changed our world. John Wiley & Sons Ltd, pp 33–49

Silva IP, de Carneiro CS, Saraiva MAF et al (2018) Antimicrobial resistance and potential virulence of Vibrio parahaemolyticus isolated from water and bivalve mollusks from Bahia, Brazil. Mar Pollut Bull 131:757–762. https://doi.org/10.1016/j.marpolbul.2018.05.007

Silvester R, Pires J, Van Boeckel TP et al (2019) Occurrence of β-lactam resistance genes and plasmid-mediated resistance among vibrios isolated from Southwest Coast of India. Microb Drug Resist 25:1306–1315. https://doi.org/10.1089/mdr.2019.0031

Smith KP, Kumar S, Varela MF (2009) Identification, cloning, and functional characterization of EmrD-3, a putative multidrug efflux pump of the major facilitator superfamily from *Vibrio cholerae* O395. Arch Microbiol 191:903–911

Someya Y, Kimura-Someya T, Yamaguchi A (2000) Role of the charge interaction between Arg(70) and Asp(120) in the Tn10-encoded metal-tetracycline/H(+) antiporter of *Escherichia coli*. J Biol Chem 275:210–214. https://doi.org/10.1074/jbc.275.1.210

Stein WD (1986) CHAPTER 6 - primary active transport systems: chemiosmosis. In: Stein WD (ed) Transport and diffusion across cell membranes. Academic Press, pp 475–612

Stein W (2012) Transport and diffusion across cell membranes. Elsevier

Sten-Knudsen O (1978) Passive transport processes. In: Tosteson DC (ed) Concepts and models. Springer, Berlin, pp 5–113

Stephen J, Lekshmi M, Ammini P et al (2022) Membrane efflux pumps of pathogenic vibrio species: role in antimicrobial resistance and virulence. Microorganisms 10:382. https://doi.org/10.3390/microorganisms10020382

Stephen J, Salam F, Lekshmi M et al (2023) The major facilitator superfamily and antimicrobial resistance efflux pumps of the ESKAPEE pathogen *Staphylococcus aureus*. Antibiotics 12:343. https://doi.org/10.3390/antibiotics12020343

Stratev D, Fasulkova R, Krumova-Valcheva G (2023) Incidence, virulence genes and antimicrobial resistance of *Vibrio parahaemolyticus* isolated from seafood. Microb Pathog 177:106050. https://doi.org/10.1016/j.micpath.2023.106050

Su X-Z, Chen J, Mizushima T et al (2005) AbeM, an H^+-coupled *Acinetobacter baumannii* multidrug efflux pump belonging to the MATE family of transporters. Antimicrob Agents Chemother 49:4362–4364. https://doi.org/10.1128/AAC.49.10.4362-4364.2005

Symmons MF, Marshall RL, Bavro VN (2015) Architecture and roles of periplasmic adaptor proteins in tripartite efflux assemblies. Front Microbiol 6:513. https://doi.org/10.3389/fmicb.2015.00513

Tabtieng R, Wattanasri S, Echeverria P et al (1989) An epidemic of *Vibrio cholerae* El Tor Inaba resistant to several antibiotics with a conjugative group C plasmid coding for type II dihydrofolate reductase in Thailand. Am J Trop Med Hyg 41:680–686. https://doi.org/10.4269/ajtmh.1989.41.680

Tan CW, Rukayadi Y, Hasan H et al (2020) Prevalence and antibiotic resistance patterns of *Vibrio parahaemolyticus* isolated from different types of seafood in Selangor, Malaysia. Saudi J Biol Sci 27:1602–1608. https://doi.org/10.1016/j.sjbs.2020.01.002

Tanabe T, Funahashi T, Nakao H et al (2003) Identification and characterization of genes required for biosynthesis and transport of the siderophore vibrioferrin in Vibrio parahaemolyticus. J Bacteriol 185:6938–6949. https://doi.org/10.1128/JB.185.23.6938-6949.2003

Tanabe T, Nakao H, Kuroda T et al (2006) Involvement of the *Vibrio parahaemolyticus pvsC* gene in export of the siderophore vibrioferrin. Microbiol Immunol 50:871–876

Tanaka Y, Hipolito CJ, Maturana AD et al (2013) Structural basis for the drug extrusion mechanism by a MATE multidrug transporter. Nature 496:247–251. https://doi.org/10.1038/nature12014

Taylor DL, Bina XR, Bina JE (2012) *Vibrio cholerae vexH* encodes a multiple drug efflux pump that contributes to the production of cholera toxin and the toxin co-regulated pilus. PLoS ONE 7:e38208

Threlfall EJ, Rowe B, Huq I (1980) Plasmid-encoded multiple antibiotic resistance in *Vibrio cholerae* El Tor from Bangladesh. Lancet 1:1247–1248. https://doi.org/10.1016/s0140-6736(80)91701-8

Towner KJ, Pearson NJ, Mhalu FS, O'Grady F (1980) Resistance to antimicrobial agents of *Vibrio cholerae* E1 Tor strains isolated during the fourth cholera epidemic in the United Republic of Tanzania. Bull World Health Organ 58:747–751

Tseng TT, Gratwick KS, Kollman J et al (1999) The rnd permease superfamily: an ancient, ubiquitous and diverse family that includes human disease and development proteins. J Mol Microbiol Biotechnol 1:107–125

Upadhyay N, Kar D, Deepak Mahajan B et al (2019) The multitasking abilities of MATE 1285 transporters in plants. J Exp Bot 70:4643–4656. https://doi.org/10.1093/jxb/erz246

Varela MF, Griffith JK (1993) Nucleotide and deduced protein sequences of the class D tetracycline resistance determinant: relationship to other antimicrobial transport proteins. Antimicrob Agents Chemother 37:1253–1258. https://doi.org/10.1128/aac.37.6.1253

Varela MF, Kumar S (2019) Strategies for discovery of new molecular targets for anti-infective drugs. Curr Opin Pharmacol 48:57–68. https://doi.org/10.1016/j.coph.2019.04.015

Varela MF, Wilson TH (1996) Molecular biology of the lactose carrier of *Escherichia coli*. Biochem Biophys Acta 1276:21–34. https://doi.org/10.1016/0005-2728(96)00030-8

Varela MF, Sansom CE, Griffith JK (1995) Mutational analysis and molecular modelling of an amino acid sequence motif conserved in antiporters but not symporters in a transporter superfamily. Mol Membr Biol 12:313–319. https://doi.org/10.3109/09687689509072433

Verma J, Bag S, Saha B et al (2019) Genomic plasticity associated with antimicrobial resistance in *Vibrio cholerae*. Proc Natl Acad Sci 116:6226–6231. https://doi.org/10.1073/pnas.1900141116

Vezzulli L, Baker-Austin C, Kirschner A et al (2020) Global emergence of environmental non-O1/O139 *Vibrio cholerae* infections linked with climate change: a neglected research field? Environ Microbiol 22:4342–4355. https://doi.org/10.1111/1462-2920.15040

Vu TTT, Hoang TTH, Fleischmann S et al (2022) Quantification and antimicrobial resistance of *Vibrio parahaemolyticus* in retail seafood in Hanoi, Vietnam. J Food Prot 85:786–791. https://doi.org/10.4315/JFP-21-444

Wade BM (2022) The blue death: cholera and reimagined community in nineteenth-century Havana. In: Venkatesan S, Chatterjee A, Lewis AD, Callender B (eds) Pandemics and epidemics in cultural representation. Springer Nature, Singapore, pp 81–103

Waldor MK, Tschäpe H, Mekalanos JJ (1996) A new type of conjugative transposon encodes resistance to sulfamethoxazole, trimethoprim, and streptomycin in *Vibrio cholerae* O139. J Bacteriol 178:4157–4165. https://doi.org/10.1128/jb.178.14.4157-4165.1996

Wei Y, Perez LJ, Ng W-L et al (2011) Mechanism of *Vibrio cholerae* autoinducer-1 biosynthesis. ACS Chem Biol 6:356–365

Weng Y, Fields EG, Bina TF et al (2021) *Vibrio cholerae* TolC is required for expression of the ToxR regulon. Infect Immunity. https://doi.org/10.1128/iai.00242-21

West IC (1980) Energy coupling in secondary active transport. Biochem Biophys Acta 604:91–126. https://doi.org/10.1016/0005-2736(80)90586-6

WHO (2022) WHO fact sheet. https://www.who.int/news-room/fact-sheets/detail/food-safety. Accessed 14 Jan 2023

Woolley RC, Vediyappan G, Anderson M et al (2005) Characterization of the *Vibrio cholerae* vceCAB multiple-drug resistance efflux operon in *Escherichia coli*. J Bacteriol 187:5500–5503. https://doi.org/10.1128/JB.187.15.5500-5503.2005

Wu C, Zhao Z, Liu Y et al (2020) Type III secretion 1 effector gene diversity among vibrio isolates from coastal areas in China. Front Cell Infect Microbiol. https://doi.org/10.3389/fcimb.2020.00301

Yaffe D, Radestock S, Shuster Y et al (2013) Identification of molecular hinge points mediating alternating access in the vesicular monoamine transporter VMAT2. Proc Natl Acad Sci 110:E1332–E1341

Yamaguchi A, Someya Y, Sawai T (1992) Metal-tetracycline/H^+ antiporter of *Escherichia coli* encoded by transposon Tn10. The role of a conserved sequence motif, GXXXXRXGRR, in a putative cytoplasmic loop between helices 2 and 3. J Biol Chem 267:19155–19162

Ye L, Zheng Z, Xu Y et al (2023) Prevalence and genetic basis of tetracycline resistance in *Vibrio parahaemolyticus* isolates recovered from food products in Shenzhen, China during 2013 to 2021. Sci Total Environ 902:166026. https://doi.org/10.1016/j.scitotenv.2023.166026

UNHCR Yemen flooding escalates spread of cholera (2023). In: UNHCR Hong Kong. https://www.unhcr.org/hk/en/23296-yemen-flooding-escalates-spread-of-cholera.html. Accessed 8 Oct 2023

Yokota T, Kuwahara S (1977) Temperature-sensitive R plasmid obtained from naturally isolated drug-resistant *Vibrio cholerae* (Biotype El Tor). Antimicrob Agents Chemother 11:13–20. https://doi.org/10.1128/AAC.11.1.13

Yuan Y, Feng Z, Wang J (2020) *Vibrio vulnificus* Hemolysin: biological activity, regulation of *vvhA* expression, and role in pathogenesis. Front Immunol 11:599439. https://doi.org/10.3389/fimmu.2020.599439

Zaidenstein R, Sadik C, Lerner L et al (2008) Clinical characteristics and molecular subtyping of *Vibrio vulnificus* illnesses, Israel.

Emerg Infect Dis 14:1875–1882. https://doi.org/10.3201/eid1412.080499

Zanetti S, Spanu T, Deriu A et al (2001) In vitro susceptibility of *Vibrio* spp. isolated from the environment. Int J Antimicrob Agents 17:407–409. https://doi.org/10.1016/S0924-8579(01)00307-7

Zhang G, Sun K, Ai G et al (2019) A novel family of intrinsic chloramphenicol acetyltransferase CATC in *Vibrio parahaemolyticus*: naturally occurring variants reveal diverse resistance levels against chloramphenicol. Int J Antimicrob Agents 54:75–79. https://doi.org/10.1016/j.ijantimicag.2019.03.012