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ORIGINAL PAPER

The Emergence of Different Bacterial Pathogens in Hospital Wastewater Samples and their Antibiotic Resistance Pattern

Asim Shahzad[1] · **Abrar Hussain Mian**[2] · **Ihtesham Ul haq**[3] · **Muhammad Azam Khan**[4] · **Matiullah**[2] · **Kashmala Ali**[5] · **Tauseef Hamid**[6]

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Abstract

The present study was designed to identify different bacterial pathogens in hospital wastewater samples and to determine their antibiotic resistant pattern against commonly used antibiotics. A total of 200 hospital wastewater samples were collected and analyzed accordingly. Of the 200 samples, only 60 yielded confirmed bacterial growth, while the remaining 140 samples showed negative results. Among 60 positive samples, a total of 7 different bacterial pathogens were isolated that included *Staphylococcus aureus* 16 (26.66%), *Escherichia coli* 14 (23.33%), *P. aeruginosa* 10 (16.66%), *S. epidermidis* 8 (13.33%), *Enterobacter* spp 7 (11.66%), *S. pyogene* 3 (5%) and *Shigella* 2 (3.33%). The antibiotic resistance pattern was checked by disc diffusion assay. All pathogens showed prominent susceptibility to Vancomycin, Cefixime, Tazobactam, and Doxycyline followed by other antibiotics, while pathogens showed noticeable resistance against Gentamycin, Amoxicillin, Tazobactam, and Tetracycline followed by other commonly used antibiotics. However, the *S. pyogenes* was the only pathogen that showed 100% resistance to Gentamycin, Tetracycline and levofloxacin, followed by *Shigella* which showed 100% resistance to Gentamycin and Tetracycline each. In particular, *S. aureus* was the most common pathogen isolated among other pathogens. The antibiotics to which bacterial pathogens showed high resistance were Gentamycin, Tetracycline, and Levofloxacin followed by other used antibiotics, while antibiotics of choice to which pathogens were prominently susceptible were Vancomycin, Cefixime, Tazobactam, and Doxycycline followed other used antibiotics. Hence, it was revealed from the present study that hospital wastewater communities are highly burdened by these antibiotic resistant bacteria and these are continuously disseminating to the outer environment which could pose an alarming situation to public health. Therefore, proper management protocols should need to be adopted to reduce the risk of exposure to such pathogens.

Keywords Wastewater · Bacterial pathogens · Antibiotic resistance pattern

Introduction

The increase of resistance development in pathogenic bacteria has become a crucial problem throughout the world due to human-created phenomenon where people have no idea of proper hygiene and immoral use of antibiotics (Khawaja et al. 2017). Hospital wastewater is the main reservoir in the colonization of resistant bacterial pathogens and acts as the source of infection to humans and the environment (Abdel-Raouf et al. 2012; Amine 2013). The main source of transmission of these pathogens is the health workers while handling different operative devices such as catheters and ventilators or through improper disposal of hospital waste materials to the effluent (Ikram et al. 2010; Khan et al. 2017). On the other hand, the direct discharge of antibiotics residues into wastewater utilizes by patients

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✉ Abrar Hussain Mian
abrar.microbio@hu.edu.pk

[1] Department of Microbiology and Biotechnology, Abasyn University, Peshawar 25000, Pakistan

[2] Department of Microbiology, Hazara University, Garden Campus, Mansehra 21300, Pakistan

[3] Department of Zoology, Hazara University, Garden Campus, Mansehra 21300, Pakistan

[4] Department of Microbiology, Government Degree College Madyan, Swat, Pakistan

[5] Department of Botany, Abdul Wali Khan University Mardan, Mardan, Pakistan

[6] University of Engineering and Technology Lahore, Lahore 54890, Pakistan

in the hospital can directly apply some selective pressure and help in the persistence of resistant bacteria (Beyene and Redaie 2011; Stalder et al. 2014). The dissemination of these resistant pathogens into the outer environment can pose an alarming situation to public health problems (Keen and Patrick 2013; Nunez and Moretton 2007; Pandey et al. 2011; Diwan et al., 2010). Bacterial pathogens most commonly present in hospital wastewater are *Staphylococcus aureus*, *Escherichia coli*, *P. aeruginosa*, *Klebsiella*, *Vibrio*, *Shigella*, and *Salmonella*, etc. (Anitha and Jayraaj 2012; Pandey et al. 2011). According to the Center for Disease Control (CDC) and the European Medicines Agency (EMA) who estimated in the latest report that in European countries the ESBL and MDR bacterial infection is liable for the death of at least 25,000 patients each year (Makky et al. 2013; Osthoff et al. 2015). This situation has also become worsened in Pakistan (Ilyas et al. 2017). From 2010 to 2013, colistin resistance was reported in *Klebsiella pneumonia* that had posed a serious threat to the public health of Pakistan (Crawford et al. 2016). The reasons observed behind acquiring MDR infection were lack of knowledge about a particular disease, improper diagnosis and medication and poor socioeconomic conditions (Sharma et al. 2015; Sethi et al. 2006). Hence, it can be said that the resistance showed by bacterial pathogens against different antibiotics can make them less effective to treat a particular disease in the society (Delgado et al. 2016). Therefore, the present study was designed to identify different resistant bacterial pathogens in wastewater samples of Lady Reading Hospital, Peshawar, Pakistan which act as a serious public health consequence while disseminating from the hospital to the outer environment.

Materials and Methods

Sample Collection and Transportation

For the present study, a total of 200 wastewater samples were gathered from different wards of Lady Reading Hospital, Peshawar, Pakistan that included operation theater, pediatrics, gynecology, ENT, orthopedics and surgical wards. The wastewater samples were collected in the 100 ml sterile high-density polyethylene (HDPE) bottles. All samples were properly labeled and maintained at 4 °C and immediately transferred under aseptic conditions to the Microbiology Laboratory at Abasyn University Peshawar, Pakistan for further processing.

Sample Processing

A 1 ml of wastewater sample was taken from each sample with the help of a pippet and was initially inoculated onto a Nutrient Agar media plate using the streak plate method and incubated for 24 h at 37 °C. Bacterial culture was obtained from each sample plate and was further cultured upon selective and enriched media included Eosin Methylene Blue (EMB) Agar, Tryptic Soy Agar (TSA), MacConkey Agar, Blood Agar, Salmonella Shigella Agar (SSA) and Cysteine lactose electrolyte deficient (CLED) agar and kept in the incubator for 24 h at 37 °C. After incubation, bacterial isolates were obtained from each plate and were sub-cultured upon the same media for pure cultured colonies.

Gram Staining

Gram staining technique was performed to differentiate gram-positive and gram-negative bacteria by studying their morphological characteristics. All slides were then observed under 100 × of the light microscope (Cheesbrough 2006).

Biochemical Tests

According to the CLSI, different biochemical tests included Oxidase, Catalase, Urease, Coagulase, Citrate, Indole, TSI and Motility test were performed for the identification and characterization of bacterial pathogens (Cheesbrough 2006; CLSI 2011).

Antibiotics Sensitivity Test

The Kirby Bauer disc diffusion method was followed to perform an antibiotic sensitivity test. A total of 10 commonly used antibiotics (Vancomycin, Gentamycin, Amoxicillin, Cefixime, Tazobactam, Tetracycline, Levofloxacin, Cefotaxime, Chloramphenicol and Doxycycline) of various concentrations were evaluated. The bacterial isolates suspension was prepared and the turbidity was adjusted according to 0.5 McFarland. Then, the prepared suspension was spread on an MHA media plate and well dried for 3 to 4 min. The typical quantity of antibiotics was evenly placed on the surface of the MHA media plate, causing a gradient of the antibiotic adjacent to the disk. The plates were incubated for 24 h at 37 °C. As a result, a bacterial growth appears on the media plate and the zones of inhibition were calculated according to the Clinical and Laboratory Standard Institute (CLSI).

Result and Discussion

The present study confirms the identification of various bacterial pathogens characteristically by performing gram staining and biochemical tests as shown in (Table 1). Detection of the bacterial pathogens that can be done through these simple inexpensive traditional methods includes selective culture media and biochemical characterization. However, it has certain limitations such as sampling error, time consumption, and monospecific detection (El-Lathy et al. 2009). Serological and molecular techniques such as ELISA and PCR have also been used for the diagnosis of bacterial pathogens in the laboratory. As it has more advantages regarding specific detection of particular pathogen and comparatively take short time. However, they are highly costly (Quan et al. 2018; Dhar and Lee 2018).

In the present study, of the 200 wastewater samples, only 60 yielded confirmed bacterial growth, while the remaining 140 samples showed negative results. Among 60 positive samples, a total of 7 different bacterial pathogens were isolated included *Staphylococcus aureus* 16 (26.66%), *Escherichia coli* 14 (23.33%), *P. aeruginosa* 10 (16.66%), *S. epidermidis* 8 (13.33%), *Enterobacter spp* 7 (11.66%), *S. pyogene* 3 (5%) and *Shigella* 2 (3.33%) (Table 2).

In comparison, the frequency of bacterial pathogens reported in treated wastewater was *Klebsiella* spp. 10 (20%), *P. aeruginosa* 8 (16%) and *S. aureus* 8 (16%) while in untreated wastewater was *Klebsiella* spp 14 (16.7%), *S. aureus* 13 (15.5%) and *P. aeruginosa* 12 (14.3%), (Asfaw et al. 2017). Another study was reported in Thailand that also identified *Vibrio* spp., *Salmonella* spp. and other bacterial pathogens (Danchaivijitr et al. 2005). The bacterial pathogens isolated from the discharge of Hawassa University Referral Hospital, Ethiopia were *Salmonella* spp., *Shigella* spp., *E. coli*, and *S. aureus* (Fekadu et al. 2015). Similarly, a large number of enteric bacteria included *S. aureus* and *P. aeruginosa* were identified in a report in India (Chitnis et al. 2000), followed by another study in Tunisia which revealed a huge amount of bacterial pathogens such as *Salmonella* that posed a serious threat to human health (Salem et al. 2011).

Table 2 Percentage distribution of various bacterial pathogens identified in Wastewater samples

Bacterial isolates	Total number	Total (%)
S. aureus	16	26.66
E. coli	14	23.33
P. aeruginosa	10	16.66
S. epidermidis	8	13.33
Enterobacter spp.	7	11.66
S. pyogene	3	5
Shigella	2	3.33
Total	60	100

In the present study, bacterial pathogens were found most susceptible to Vancomycin, Cefixime, Tazobactam and Doxycycline followed by other antibiotics, while, pathogen such as *S. pyogenes* was (100%) resistant to Gentamycin, Tetracycline and levofloxacin, followed by *Shigella* which was (100%) resistant to Gentamycin and Tetracycline. In general, bacterial pathogens mostly showed noticeable resistance against Gentamycin, Amoxicillin, Tazobactam, and Tetracycline followed by other commonly used antibiotics (Figs. 1, 2, 3, 4, 5, 6, and 7).

In a similar study, a total of 115 bacterial isolates were identified in the hospital wastewater and waste dump soils. All pathogens showed resistance to Amoxycillin, Tetracycline, Gentamycin, Erythromycin, and Chloramphenicol. However, pathogens isolated from wastewater samples of which 4 (66.7%) showed susceptibility to Zinnacef, 3 (50%) to Pefloxacin and Nitrofurantoin and 2 (33.3%) to Ciprofloxacin (Adieze et al. 2015). Similarly, in the last discharge of wastewater purification plant in India, high resistance of bacterial pathogens was observed against cotrimoxazole, tetracycline, ampicillin, ampicillin-clavulanic acid, and cephalosporins (1st, 2nd, and 3rd generation) (Katouli et al. 2012).

Followed by another study in Alexandria, Egypt who isolated ESBL producing pathogens from the wastewater treatment process that causing a serious threat of spreading to the outer surroundings (Amine 2013). Likewise, in the

Table 1 Biochemical characteristics of different isolated bacterial pathogens shows as + = Positive, − = Negative, +/− = Variable, A/A = Acid/Acid reaction (Yellow in color), Empty = test not Performed

Name of isolates	Oxidase	Catalase	Urease	Coagulase	Citrate	Indole	TSI	Motility
S. aureus	−	+	+	+	+	−	A/A	Non-motile
E. coli	−	+	−	−	−	+	A/A	Motile
P. aeruginosa	+	+	−	−	+	−		Motile
S. epidermidis	−	+	+	−	−			Non-motile
Enterobacter spp.	_	+	_		+	_		Motile
S. pyogene		−	−					Non-motile
Shigella	−	−	−		−	+/−	Alkali/Acid	Non-motile

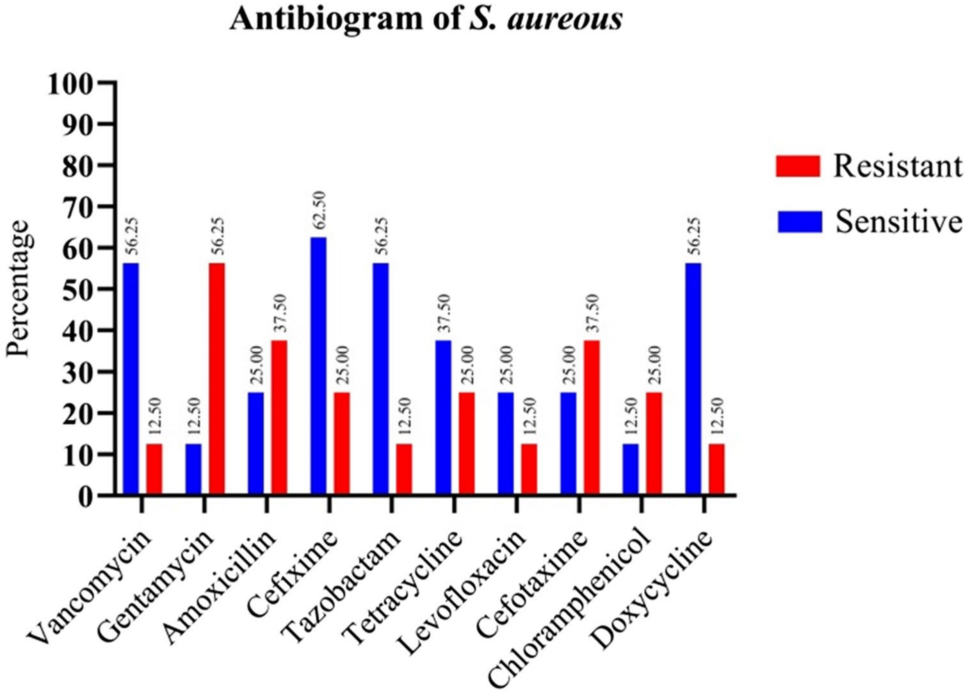


Fig. 1 Antibiogram of *S. aureous* isolated from hospital wastewater samples

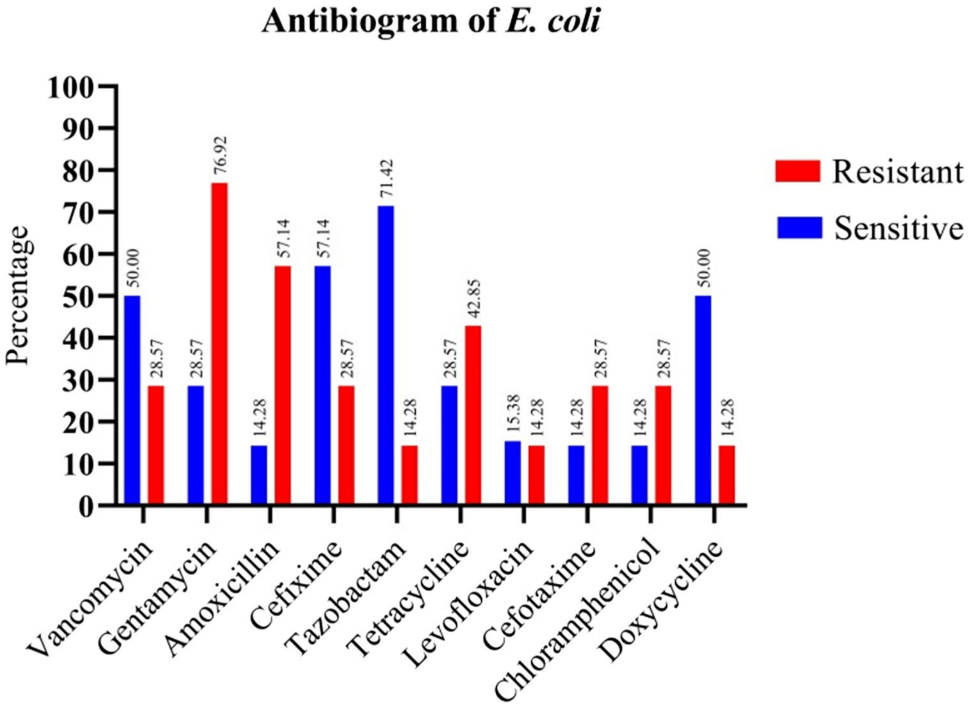


Fig. 2 Antibiogram of *E. coli* isolated from hospital wastewater samples

South Africa province, Eastern Cape (Alice), highly resistant bacterial isolates were identified in the final discharge of wastewater purification plant (Iweriebor et al., 2015), followed by similar results reported in European countries (Blanch et al. 2003; Servais and Passerat 2009).

Data about the study of hospital wastewater analysis in Pakistan is very rare due to the existence of little information about its characteristics. This is due to very little concentration being given to the proper management of hazardous waste (Meo et al. 2014).

A study in 8 hospitals of Faisalabad City, Pakistan observed that about 90% of healthcare workers were not trained enough to manage hospital hazardous waste, while 80% of the staff were not even notified from the

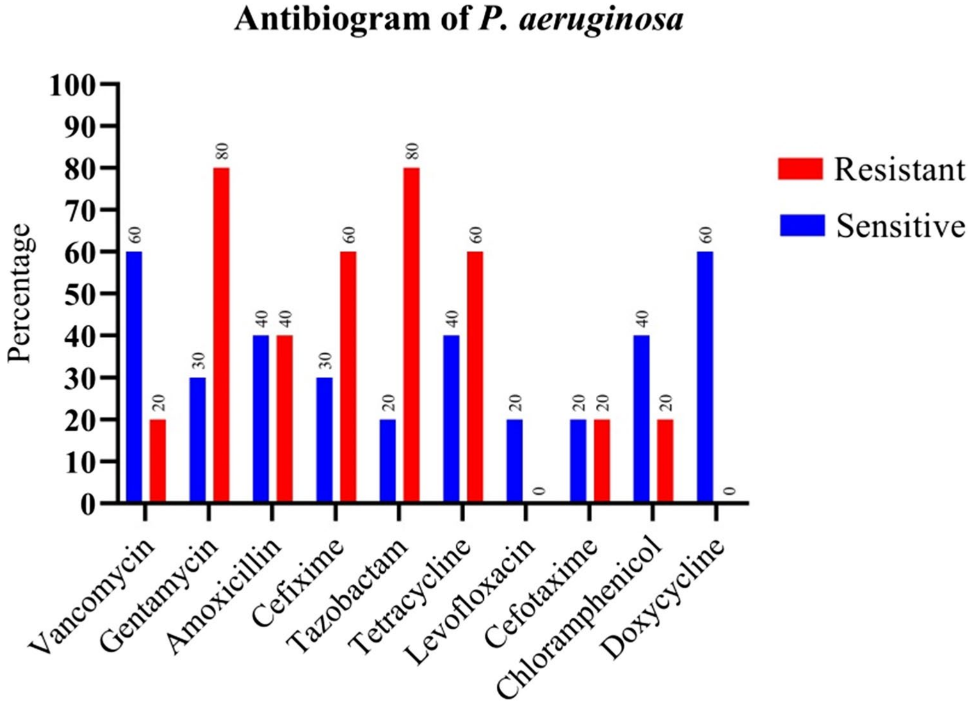


Fig. 3 Antibiogram of *P. aeruginosa* isolated from hospital wastewater samples

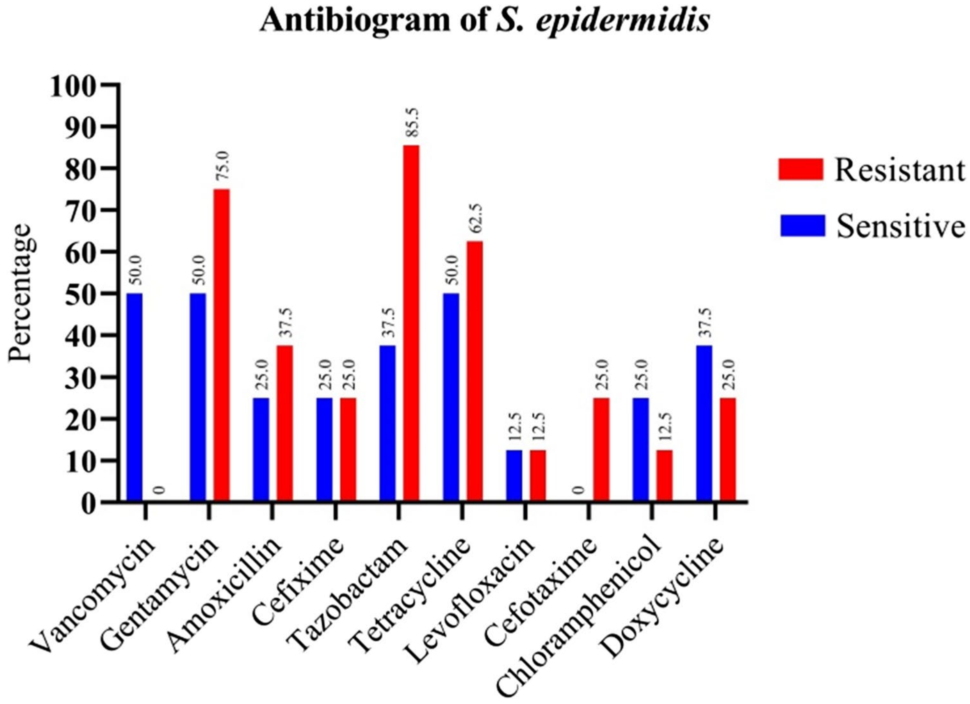


Fig. 4 Antibiogram of *S. epidermidis* isolated from hospital wastewater samples

Pakistan Environmental Protection Agency side. There were no such personnel protective equipment procedures followed by sanitary works for the transportation of waste. In short, they were unaware of the proper management and disposal of hospital waste (Anwar et al. 2013). Another study conducted in 10 large hospitals of District Rawalpindi and Islamabad revealed that there were no such proper practices of segregation followed at the time of generation. This could be attributed to a lack of proper knowledge in healthcare and other staff members about waste management. Hence, it was suggested that proper awareness in healthcare workers could ultimately enhance the practice of proper management of hospital risk waste (Meo et al. 2014).

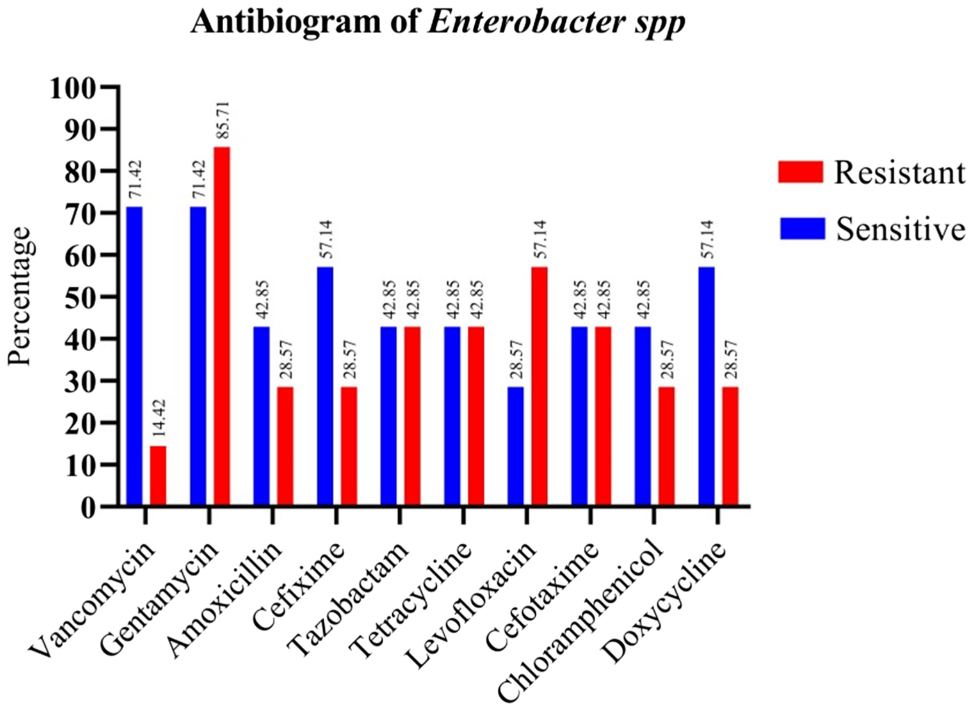


Fig. 5 Antibiogram of *Enterobacter* isolated from hospital wastewater samples

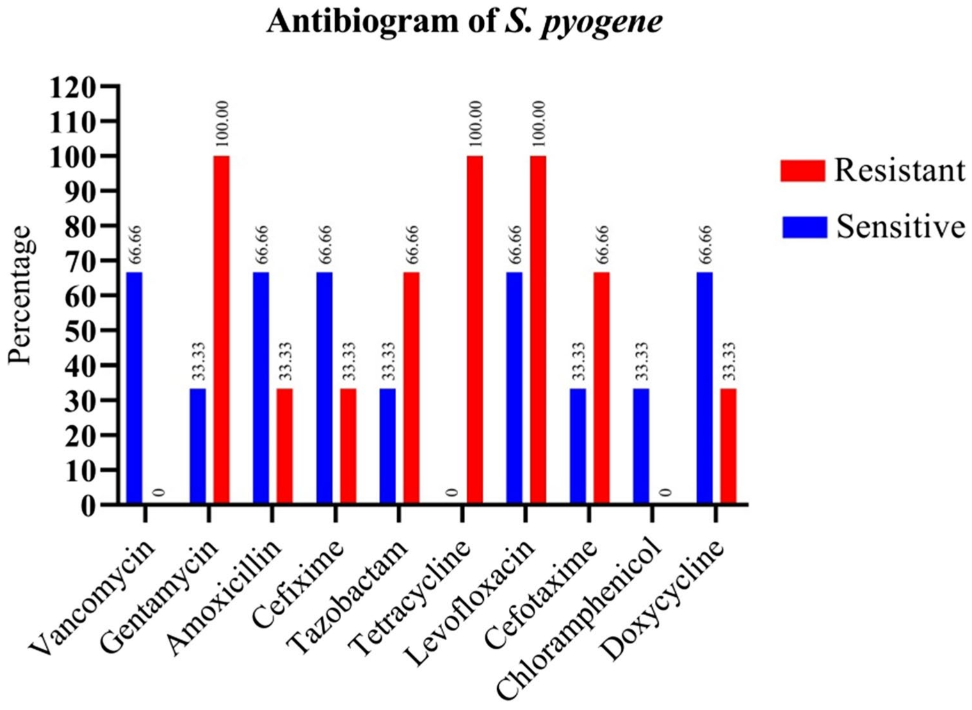
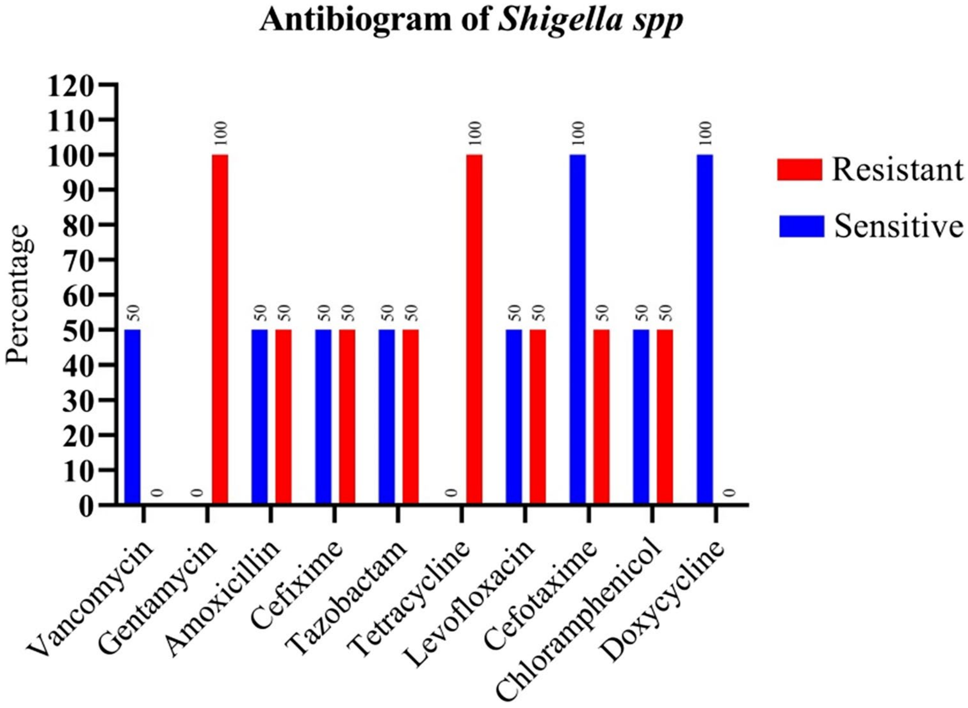


Fig. 6 Antibiogram of *S. pyogenes* isolated from hospital wastewater samples

The present study observed that hospital wastewater acts as the main reservoir for the growth of various resistance bacterial pathogens. The antibiotic residues used in different wards of hospitals for the treatment purpose could directly flush into the wastewater system, as a result, they restrain the bacterial growth which persists resistance to antibiotics. The continuous discharge of these harmful pathogens that carry antibiotic resistance genes to the surroundings can cause serious public health consequences (Asfaw et al. 2017; Efaq et al. 2015; Okojokwu et al. 2014). Therefore, to overcome such a situation, different advanced strategies are required for wastewater purification to prevent the adverse effects of pollutants before spreading into the atmosphere. The analysis of bacterial pathogens in wastewater systems is the major

Fig. 7 Antibiogram of *Shigella* spp. isolated from hospital wastewater samples



step to assess the pollution load and to develop different treatment methodologies to prevent environmental health.

Conclusion

The present study observed that *Staphylococcus aureus* was the most prominent pathogen 16 (26.66%) isolated from 200 hospital wastewater samples, followed by other bacterial pathogens including *E. coli*, *P. aeruginosa*, *S. epidermidis*, *Enterobacter* spp, *S. pyogene* and *Shigella.* Pathogens showed noticeable resistance to Gentamycin, Tetracycline, and levofloxacin followed by Amoxicillin and Tazobactam. While pathogens showed maximum susceptibility to Vancomycin, Cefixime, Tazobactam, and Doxycycline followed by other antibiotics which were concluded as antibiotics of choice which inhibiting the growth of bacterial pathogens. The presence of a huge amount of antibiotic resistant pathogens in the wastewater system of the hospital and its continuous dissemination to the outer surroundings could pose a serious threat to public health which should need to devise proper management protocols for preventive measures.

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Author contribution AS helped in conceptualization, methodology, data processing and writing - original draft manuscript. IU, MAK and Matiullah helped in data collection. KA and TH performed formal analysis. AHM helped in writing, critical review, editing and submission of the draft manuscript. All the authors read and approved the final version of the draft manuscript

Data Availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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