Chapter 3
Factors Affecting Production of Outer Membrane Vesicles

Abstract The production of outer membrane vesicles (OMVs) by Gram-negative bacteria is influenced by many different factors. Pathogenic bacteria produce more OMVs than the non-pathogenic ones and OMVs are also produced within the infected hosts. The amount of OMVs produced under different growth conditions varies, and the structure of LPS on the outer membrane significantly influences OMV production. Bacteria treated with antibiotics such as gentamicin produce numerous OMVs that are different from the native OMVs in structure and chemical composition. Similarly, bacteria under stress also produce more OMVs. Thus the OMVs are produced to favor the growth and survival of the parent bacteria under challenging conditions.

Keywords Natural and unnatural OMVs · Pathogenic and non-pathogenic bacteria · Growth conditions · LPS structure · Infected host · Antibiotic treatment · Stress response

3.1 Natural and Unnatural OMVs

Outer membrane vesicles (OMVs) are produced by Gram-negative bacteria, in general, during their active growth and not when they undergo lysis and death (Chatterjee and Das 1966, 1967). In fact, the OMVs have been found to contain newly synthesized proteins and are produced without concomitant bacterial lysis (Ellis and Kuehn 2010; McBroom et al. 2006; Mug-Opstelten and Witholt 1978; Zhou et al. 1998). However, OMVs or OMV-like particles have also been produced by certain drastic treatments (detergent, antibiotics etc.) or during abnormal or unbalanced growth in nutritionally deficient media or after artificial treatment (sonication, etc.) of bacteria. These OMVs or better OMV-like particles

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are compositionally different from naturally produced OMVs and they mostly contain materials leaking from bacteria undergoing lysis. It is proposed that these OMVs be termed unnatural OMVs vis-à-vis the natural ones formed by bacteria growing normally in a nutritionally rich culture medium or within the infected host. Genetic studies by generating transposon insertion mutants of *Escherichia coli* revealed that vesiculation or OMV production is not a consequence of bacterial lysis or disintegration of the bacterial envelope and cannot be correlated with membrane instability, and that vesiculation is a process important in the growth of Gram-negative bacteria (McBroom et al. 2006). Accordingly, the unnatural OMVs are discussed briefly as and when required for relevance only.

3.2 Pathogenic and Nonpathogenic Bacteria

OMVs are produced by both pathogenic and nonpathogenic species of Gram-negative bacteria (Beveridge 1999; Chatterjee and Das 1966, 1967; Kadurugamuwa and Beveridge 1997; Li et al. 1998; Mayrand and Grenier 1989). The different bacterial species that have already been demonstrated to release OMVs include *E. coli* (Gankema et al. 1980; Hoekstra et al. 1976), *Shigella spp*. (Dutta et al. 2004; Kadurugamuwa and Beveridge 1999), *Neisseria spp*. (Devoe and Gilchrist 1973; Dorward and Garon 1989; Dorward et al. 1989), *Pseudomonas aeruginosa* (Kadurugamuwa and Beveridge 1995), *Vibrio spp*. (Chatterjee and Das 1966, 1967; Iwanaga and Naito 1979, 1980; Kondo et al. 1993), *Helicobacter pylori* (Fiocca et al. 1999), *Salmonella spp*. (Vesy et al. 2000; Wai et al. 2003), *Brucella melitensis* (Gamazo and Moriyon 1987), *Bacteroides* (including *Porphyromonas*) *spp*. (Grenier and Mayrand 1987; Mayrand and Holt 1988; Zhou et al. 1998), *Borrelia burgdorferi* (Shoberg and Thomas 1993), and *Actinobacillus actinomycetemcomitans* (Nowotny et al. 1982). In general, pathogenic bacteria produce more vesicles than the corresponding nonpathogenic ones (Lai et al. 1981; Wai et al. 1995). In fact, Enterotoxigenic *E. coli* cells were found to produce tenfold more vesicles than their corresponding nonpathogenic ones (Horstman and Kuehn 2002)**.** Similarly the pathogenic leukotoxic strains of *A. actinomycetemcomitans* were shown to produce 25-fold more vesicles than their corresponding nonpathogenic ones (Lai et al. 1981). *E. coli* strains bearing a mutation in *hns*, a virulence regulatory factor, produced threefold more vesicles (Horstman and Kuehn 2002). This evidence gives credence to the idea that vesicle production is utilized by the pathogenic bacteria to disseminate virulence factors and gain better survival in the host. Similarly, nonpathogenic bacteria can also take recourse to vesicle production for improving survival by releasing different toxic compounds, such as toluene, and by aiding in the release or removal of the attacking phages (Kobayashi et al. 2000; Loeb 1974; Loeb and Kilner 1978).

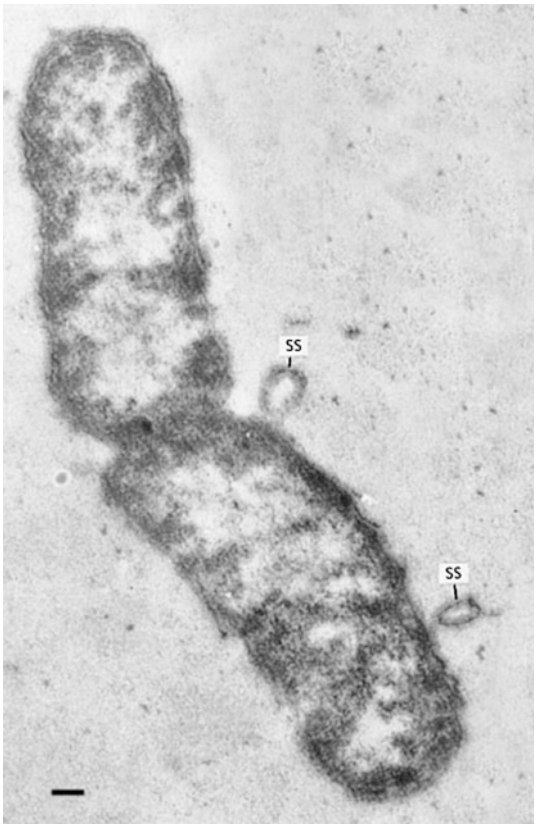

Fig. 3.1 A dividing *V. cholerae* cell, thin-sectioned, stained with potassium permanganate and electron micrographed. Saclike structures (*SS*) or OMVs formed by the bulged-out cell wall portion are presumably ready to be pinched off. Bar represents 0.1μm. From (Chatterjee and Das 1967)

3.3 Bacterial Growth Conditions

Gram-negative bacteria growing (1) in liquid culture (Chatterjee and Das 1966, 1967; Wai et al. 1995), (2) on solid growth media (Tetz et al. 1990; Unal et al. 2010), (3) in biofilms (Schooling and Beveridge 2006; Unal et al. 2010; Yonezawa et al. 2009), and (4) within hosts (Avakian et al. 1972; Fiocca et al. 1999; Galka et al. 2008; Stephens et al. 1982) produce OMVs for various purposes. OMVs are produced more actively during the exponential growth phase (Chatterjee and Das 1966, 1967) and during division at the site of division and elsewhere (Fig. 3.1) (Chatterjee and Das 1967; Deatherage et al. 2009; Kuehn and Kesty 2005). During the resting or stationary phase of growth, OMVs are not produced (Chatterjee and Das 1967) or are produced in a very limited way (Bauman and Kuehn 2006; Hoekstra et al. 1976). It may be that during the resting phase of bacterial population growth, some individual bacteria may still remain in the logarithmic phase and produce OMVs. This evidence is again consistent with the idea that OMV production is an inherent property of the bacteria and is produced for some purposes in favor of the actively growing parent bacteria.

3.4 Impact of LPS Structure

The structure of the antigenic LPS chains projecting outward from the bacterial surface has a significant effect on the formation of OMVs. There are bacterial spp. (e.g., *P. aeruginosa* strain PAO1) that express two types of LPS chains and produce vesicles enriched in the highly charged and longer "B-band" form (Beveridge et al. 1997; Kadurugamuwa and Beveridge 1995; Nguyen et al. 2003). Such enrichment happens probably because charge–charge repulsion takes place in the regions of the OM containing adjacent B-band LPS molecules leading to local deformation and budding of the OM (Kadurugamuwa and Beveridge 1996; Li et al. 1996). Thus, *P. aeruginosa* strain PAO1 was shown to produce more B-band LPS and an increase in vesiculation when grown under oxygen stress conditions (Sabra et al. 2003). Mutants of the *Salmonella* and *P. aeruginosa* strains having no LPS O-antigen side chain produced more OMVs (Meadow et al. 1978; Smit et al. 1975). On the other hand, mutations in the core region of LPS were shown to be associated with decreased expression of outer membrane proteins (OMPs) (Ames et al. 1974; Schnaitman and Klena 1993; Smit et al. 1975). It was thus interpreted that the vesiculation phenotypes produced in LPS core mutants were the result of alteration in OMP composition and hence were the indirect effects (Meadow et al. 1978) of core mutation. A typical defense strategy of bacteria is to alter expression of LPS O-antigen to evade the host response (Lerouge and Vanderleyden 2002; Pier 2000). The presence and type of LPS O-antigen as well as the indirect effect of oxygen stress on the LPS structure may influence the physical ability of the membrane to bulge and initiate the formation of vesicles.

3.5 Vesicle Production Within the Infected Host

Gram-negative bacteria have been shown to produce OMVs while present within the host system (Ellis and Kuehn 2010) and in a variety of environments. Presence of antibiotics, availability of iron, LPS phenotype switching, and oxygen stress are some of the factors the bacteria face within the infected host and these conditions influence vesicle production there (Kuehn and Kesty 2005). Perhaps the earliest evidence in this respect was produced by Avakyan and co-workers by electron microscopy of the biopsied sections of small intestine mucosa in patients with cholera (Avakian et al. 1972) and then by Halhoul and Colvin in their study on the ultrastructure of plaque attached to human gingiva (Halhoul and Colvin 1975). However, both the composition and production of vesicles within the host systems depend on the environmental factors that the bacteria face within the host.

H. pylori are known to colonize the stomach and cause peptic ulcer and even cancer. These colonizing bacteria were shown by electron microscopy to produce vesicles that bind to gastric cells (Fiocca et al. 1999; Keenan et al. 2000; Keenan and Allardyce 2000). These bacteria experience different levels of iron within the

host. These vesicles contained the vacuolating cytotoxin, VacA, and they were very similar to those produced by *H. pylori* in vitro (Fiocca et al. 1999; Keenan et al. 2000). It was shown that the vesicles derived from *H. pylori* were in contact with the intestinal epithelial cells (Fiocca et al. 1999; Heczko et al. 2000; Keenan et al. 2000). However, growth of these bacteria in iron-limiting conditions reduces VacA and increases the concentration of proteases within the vesicles while maintaining at the same time the vesicle production level (Keenan and Allardyce 2000). The OMVs from a highly vesiculating strain of *Neisseria meningitidis* causing a fatal septic infection in humans were the causative factors of a high level of endotoxins in the cells (Namork and Brandtzaeg 2002). Mouse fibroblast cells infected with *Chlamydia trachomatis* or *Chlamydia psittaci* were shown to contain vesicles (Stirling and Richmond 1980). When the *B. burgdorferi* isolate (causative factor of Lyme disease) was incubated with human skin for about 24 h, vesicles could be detected after the organisms invaded the dermis (Beermann et al. 2000).

Different fluids isolated from the infected hosts also contained vesicles. This indicated that the vesicles could migrate to sites at a distance from the point of infection. *N. meningitidis* along with the vesicles they released were found in the cerebrospinal fluid from a patient with meningitis and blood from a patient who died from meningitis (Bjerre et al. 2000; Brandtzaeg et al. 1992; Craven et al. 1980; Namork and Brandtzaeg 2002; Stephens et al. 1982). Similarly, vesiculating *Borrelia* and free vesicles were detected in the blood and urine of *B. burgdorferi*-infected mice and also ticks (Dorward et al. 1991). Also, *Salmonella typhimurium* organisms were shown to produce vesicles when growing intracellularly and also in the in vitro culture fluid (Bergman et al. 2005; Garcia-del Portillo et al. 1997; Vesy et al. 2000).

These findings strongly support the idea that vesicle production takes place within an infected host and that the vesicles are found surrounding the parent bacteria and/or in contact with the host cells. They are also available in different fluids collected from the infected patients. The vesicle production within the infected host is thus an inherent property of the bacteria. But to what extent these vesicles are directly responsible for spreading infection within the host by acting in "self-defense" or by killing the host or other co-colonizing bacterial cells and if this is true, whether they can be targeted or selectively destroyed to save the patient remains an important subject for further studies.

3.6 Antibiotic Treatment and Vesicle Formation

Treatment of bacteria with antibiotics often leads to unnatural vesicle production. Several aspects of vesiculation were shown to be affected by antibiotic treatment and the response differed with the antibiotic. Of the different antibiotics available, gentamicin in particular has been studied in detail. *P. aeruginosa* on treatment with gentamicin produces at least three fold more vesicles and the structure of these vesicles is significantly different from those produced by the same bacteria

under normal growth conditions (Kadurugamuwa and Beveridge 1998). The gentamicin-induced vesicles serve an important purpose, that is, they fuse to host cells infected by pathogenic bacteria, deliver the antibiotic, gentamicin, in the cytosol and thereby kill the intracellular bacteria (Kadurugamuwa and Beveridge 1998). The gentamicin-induced vesicles are larger in size *vis-à-vis* those produced by normally growing bacteria and contain in addition to the OM and periplasmic components, some components of the IM and the cytosol (Kadurugamuwa and Beveridge 1995). In addition, the gentamicin-induced vesicles of *P. aeruginosa* are not enriched in the B-band LPS that are found in native vesicles. Although the gentamicin-induced vesicles appear to be produced by a different mechanism, they served the purpose of elucidating the fusogenic capacity of the vesicles and their ability to interact with neighboring cells. The gentamicin-induced *P. aeruginosa* vesicles were also bacteriolytic, a mechanism that helped the bacteria in securing a niche in a competitive microbial environment (Kadurugamuwa and Beveridge 1996; Allan and Beveridge 2003). In *Shigella dysenteriae*, treatment with mitomycin C caused production of Shiga toxin and also increased production of OMVs of greater sizes and toxicity (Dutta et al. 2004). Treatment with some other antibiotics produced different responses (Dutta et al. 2004). Treatment with the antibiotics, fosfomycin, ciprofloxacin, and norfloxacin, did not have any significant effect on vesicle production or toxicity (Kuehn and Kesty 2005).

3.7 Stress Response and OMV Production

Envelope stress is produced by several factors including impairment of protein folding in the periplasm. Increased production of OMVs is a significant one among different modes of bacterial stress response. There are several mechanisms of invoking stress responses by Gram-negative bacteria facing different stressors (Raivio 2005). The σ^E is one of the different stress response pathways and is activated by events or mutations that lead to alterations in OMP biogenesis including misfolding of proteins in the periplasm. Protein misfolding in the periplasm leads to the activation of several events: (1) the membrane-bound antisigma factor, RseA, is cleaved by the protease, DegS; (2) normally another periplasmic regulatory molecule, RseB, protects RseA from cleavage in the absence of inducing signals; (3) the degradation of RseA leads to the release of σ^E into the cytoplasm and the transcriptional activation of a set of genes that include many involved in OMP and outer-membrane biogenesis (Raivio 2005). Two of the genes, *degS* and *rseA*, code for stress signal transmitters in the σ^E stress-response pathway, and another gene, *degP*, codes for a downstream effector. Activity of σ^E is essential under both stress and nonstress conditions, and in addition to its role in monitoring and maintaining OMPs in the face of adverse conditions, σ^E plays other key physiological roles (De Las Penas et al. 1997). The Cpx is another envelope stress-response pathway that appears to maintain envelope protein folding status in the presence of adverse conditions (Raivio 2005). McBroom et al. (2006)

revealed that vesiculation levels were altered by mutation of an envelope stress-response pathway. Some disruption of genes in this pathway was known to result in low σ^E activity, whereas others caused no change or even hyperactivation of the σ^E response (Alba and Gross 2004). Interestingly, all the σ^E pathway mutants of *E. coli* obtained by McBroom et al. (2006) caused increased vesiculation. The authors proposed that impairment and hyperactivation of the σ^E pathway perhaps resulted in accumulation of materials in the cell envelope, which induced heightened vesiculation. They studied the relation between vesiculation and activation of the σ^E pathway and thought that vesiculation under stressing conditions possibly occurred via a mechanism that differed from the typical vesiculation process.

McBroom and Kuehn presented data that revealed another novel stress response mechanism of Gram-negative bacteria, the release of outer membrane vesicles (McBroom and Kuehn 2007). By using an elegant genetic method (transposon mutagenesis screen) and several *E. coli* mutants, they showed that vesicle production is not directly correlated with σ^E pathway activity; that is there are other means of regulating the vesiculation level. The significant findings of their study include: (1) vesiculation increases in response to impairment of the σ^E pathway, (2) vesiculation is regulated by the level of protein accumulation in the envelope periplasm, (3) mutations that cause increased vesiculation improve bacterial survival under stress including accumulation of toxic misfolded proteins, (4) vesiculation is a distinctly independent stress response, (5) vesiculation does not involve any significant loss of membrane integrity, and (6) the vesiculation process can act to selectively eliminate unwanted materials such as misfolded proteins in the periplasm.

Involvement of a different entity in the process of OMV formation was presented (Song et al. 2008). A small noncoding s-RNA gene, *vrrA*, was discovered in *V. cholerae* O1 strain A1552. The corresponding VrrA RNA (140 nt) was found to repress the *ompA* translation. It was shown that the expression of the *vrrA* gene required the membrane stress factor σ^E, suggesting that *vrrA* acted on *ompA* in response to periplasmic protein-folding stress. The OmpA levels were again found to correlate inversely with the number of OMVs produced and that VrrA increased OMV production comparable to loss of OmpA. VrrA was thus shown to control OMV production. It was proposed that VrrA acted as a regulator mediating σ^E related stress.

References

Alba BM, Gross CA (2004) Regulation of the *Escherichia coli* sigma-dependent envelope stress response. Mol Microbiol 52:613–619

Allan ND, Beveridge TJ (2003) Gentamicin delivery to *Burkholderia cepacia* group IIIa strains via membrane vesicles from Pseudomonas aeruginosa PAO1. Antimicrob Agents Chemother 47:2962–2965

Ames GF, Spudich EN, Nikaido H (1974) Protein composition of the outer membrane of *Salmonella typhimurium*: effect of lipopolysaccharide mutations. J Bacteriol 117:406–416

Avakian AA, Sinel'nikova MP, Pereverzev NA, Gurskii IuN (1972) Electron microscopic study of biopsied sections of small intestine mucosa in patients with cholera and characteristics of the ultrastructure of causative agents of cholera in relation to toxinogenesis. Zh Mikrobiol Epidemiol Immunobiol 49:86–92

Bauman SJ, Kuehn MJ (2006) Purification of outer membrane vesicles from *Pseudomonas aeruginosa* and their activation of an IL-8 response. Microbes Infect 8:2400–2408

Beermann C, Wunderli-Allenspach H, Groscurth P, Filgueira L (2000) Lipoproteins from *Borrelia burgdorferi* applied in liposomes and presented by dendritic cells induce CD8(+) T-lymphocytes in vitro. Cell Immunol 201:124–131

Bergman MA, Cummings LA, Barrett SL, Smith KD, Lara JC, Aderem A, Cookson BT (2005) CD4 + T cells and toll-like receptors recognize *Salmonella* antigens expressed in bacterial surface organelles. Infect Immun 73:1350–1356

Beveridge TJ (1999) Structures of gram-negative cell walls and their derived membrane vesicles. J Bacteriol 181:4725–4733

Beveridge TJ, Makin SA, Kadurugamuwa JL, Li Z (1997) Interactions between biofilms and the environment. FEMS Microbiol Rev 20:291–303

Bjerre A, Brusletto B, Rosenqvist E, Namork E, Kierulf P et al (2000) Cellular activating properties and morphology of membrane-bound and purified meningococcal lipopolysaccharide. J Endotoxin Res 6:437–445

Brandtzaeg P, Bryn K, Kierulf P, Ovstebo R, Namork E, Aase B, Jantzen E (1992) Meningococcal endotoxin in lethal septic shock plasma studied by gas chromatography, mass-spectrometry, ultracentrifugation, and electron microscopy. J Clin Invest 89:816–823

Chatterjee SN, Das J (1966) Secretory activity of *Vibrio cholerae* as evidenced by electron microscopy. In: Uyeda R (ed) Electron Microscopy 1966. Maruzen Co. Ltd, Tokyo

Chatterjee SN, Das J (1967) Electron microscopic observations on the excretion of cell-wall material by *Vibrio cholerae*. J Gen Microbiol 49:1–11

Craven DE, Peppler MS, Frasch CE, Mocca LF, McGrath PP, Washington G (1980) Adherence of isolates of *Neisseria meningitidis* from patients and carriers to human buccal epithelial cells. J Infect Dis 142:556–568

De Las Penas A, Connolly L, Gross CA (1997) SigmaE is an essential sigma factor in *Escherichia coli*. J Bacteriol 179:6862–6864

Deatherage BL, Lara JC, Bergsbaken T, Rassoulian Barrett SL, Lara S, Cookson BT (2009) Biogenesis of bacterial membrane vesicles. Mol Microbiol 72:1395–1407

Devoe IW, Gilchrist JE (1973) Release of endotoxin in the form of cell wall blebs during in vitro growth of *Neisseria meningitidis*. J Exp Med 138:1156–1167

Dorward DW, Garon CF (1989) DNA-binding proteins in cells and membrane blebs of *Neisseria gonorrhoeae*. J Bacteriol 171:4196–4201

Dorward DW, Garon CF, Judd RC (1989) Export and intercellular transfer of DNA via membrane blebs of *Neisseria gonorrhoeae*. J Bacteriol 171:2499–2505

Dorward DW, Schwan TG, Garon CF (1991) Immune capture and detection of *Borrelia burgdorferi* antigens in urine, blood, or tissues from infected ticks, mice, dogs, and humans. J Clin Microbiol 29:1162–1170

Dutta S, Iida K, Takade A, Meno Y, Nair GB, Yoshida S (2004) Release of Shiga toxin by membrane vesicles in *Shigella dysenteriae* serotype 1 strains and in vitro effects of antimicrobials on toxin production and release. Microbiol Immunol 48:965–969

Ellis TN, Kuehn MJ (2010) Virulence and immunomodulatory roles of bacterial outer membrane vesicles. Microbiol Mol Biol Rev 74:81–94

Fiocca R, Necchi V, Sommi P, Ricci V, Telford J, Cover TL, Solcia E (1999) Release of *Helicobacter pylori* vacuolating cytotoxin by both a specific secretion pathway and budding of outer membrane vesicles. Uptake of released toxin and vesicles by gastric epithelium. J Pathol 188:220–226

Galka F, Wai SN, Kusch H, Engelmann S, Hecker M et al (2008) Proteomic characterization of the whole secretome of *Legionella pneumophila* and functional analysis of outer membrane vesicles. Infect Immun 76:1825–1836

Gamazo C, Moriyon I (1987) Release of outer membrane fragments by exponentially growing *Brucella melitensis* cells. Infect Immun 55:609–615

Gankema H, Wensink J, Guinee PA, Jansen WH, Witholt B (1980) Some characteristics of the outer membrane material released by growing enterotoxigenic *Escherichia coli*. Infect Immun 29:704–713

Garcia-del Portillo F, Stein MA, Finlay BB (1997) Release of lipopolysaccharide from intracellular compartments containing *Salmonella typhimurium* to vesicles of the host epithelial cell. Infect Immun 65:24–34

Grenier D, Mayrand D (1987) Functional characterization of extracellular vesicles produced by *Bacteroides gingivalis*. Infect Immun 55:111–117

Halhoul N, Colvin JR (1975) The ultrastructure of bacterial plaque attached to the gingiva of man. Arch Oral Biol 20:115–118

Heczko U, Smith VC, Mark Meloche R, Buchan AM, Finlay BB (2000) Characteristics of *Helicobacter pylori* attachment to human primary antral epithelial cells. Microbes Infect 2:1669–1676

Hoekstra D, van der Laan JW, de Leij L, Witholt B (1976) Release of outer membrane fragments from normally growing *Escherichia coli*. Biochim Biophys Acta 455:889–899

Horstman AL, Kuehn MJ (2002) Bacterial surface association of heat-labile enterotoxin through lipopolysaccharide after secretion via the general secretory pathway. J Biol Chem 277:32538–32545

Iwanaga M, Naito T (1979) Morphological changes of *Vibrio cholerae* during toxin production. Trop Med 21:187–196

Iwanaga M, Naito T (1980) Toxin production and morphological changes of *Vibrio cholerae* in the medium for inducing pleomorphism. Trop Med 22:61–68

Kadurugamuwa JL, Beveridge TJ (1995) Virulence factors are released from *Pseudomonas aeruginosa* in association with membrane vesicles during normal growth and exposure to gentamicin: a novel mechanism of enzyme secretion. J Bacteriol 177:3998–4008

Kadurugamuwa JL, Beveridge TJ (1996) Bacteriolytic effect of membrane vesicles from *Pseudomonas aeruginosa* on other bacteria including pathogens: conceptually new antibiotics. J Bacteriol 178:2767–2774

Kadurugamuwa JL, Beveridge TJ (1997) Natural release of virulence factors in membrane vesicles by *Pseudomonas aeruginosa* and the effect of aminoglycoside antibiotics on their release. J Antimicrob Chemother 40:615–621

Kadurugamuwa JL, Beveridge TJ (1998) Delivery of the non-membrane-permeative antibiotic gentamicin into mammalian cells by using *Shigella flexneri* membrane vesicles. Antimicrob Agents Chemother 42:1476–1483

Kadurugamuwa JL, Beveridge TJ (1999) Membrane vesicles derived from *Pseudomonas aeruginosa* and Shigella flexneri can be integrated into the surfaces of other gram-negative bacteria. Microbiology 145(Pt 8):2051–2060

Keenan JI, Allardyce RA (2000) Iron influences the expression of *Helicobacter pylori* outer membrane vesicle-associated virulence factors. Eur J Gastroenterol Hepatol 12:1267–1273

Keenan J, Day T, Neal S, Cook B, Perez-Perez G, Allardyce R, Bagshaw P (2000) A role for the bacterial outer membrane in the pathogenesis of *Helicobacter pylori* infection. FEMS Microbiol Lett 182:259–264

Kobayashi H, Uematsu K, Hirayama H, Horikoshi K (2000) Novel toluene elimination system in a toluene-tolerant microorganism. J Bacteriol 182:6451–6455

Kondo K, Takade A, Amako K (1993) Release of the outer membrane vesicles from *Vibrio cholerae* and *Vibrio parahaemolyticus*. Microbiol Immunol 37:149–152

Kuehn MJ, Kesty NC (2005) Bacterial outer membrane vesicles and the host-pathogen interaction. Genes Dev 19:2645–2655

Lai CH, Listgarten MA, Hammond BF (1981) Comparative ultrastructure of leukotoxic and non-leukotoxic strains of *Actinobacillus actinomycetemcomitans*. J Periodontal Res 16:379–389

Lerouge I, Vanderleyden J (2002) O-antigen structural variation: mechanisms and possible roles in animal/plant-microbe interactions. FEMS Microbiol Rev 26:17–47

Li Z, Clarke AJ, Beveridge TJ (1996) A major autolysin of *Pseudomonas aeruginosa*: subcellular distribution, potential role in cell growth and division and secretion in surface membrane vesicles. J Bacteriol 178:2479–2488

Li Z, Clarke AJ, Beveridge TJ (1998) Gram-negative bacteria produce membrane vesicles which are capable of killing other bacteria. J Bacteriol 180:5478–5483

Loeb MR (1974) Bacteriophage T4-mediated release of envelope components from *Escherichia coli*. J Virol 13:631–641

Loeb MR, Kilner J (1978) Release of a special fraction of the outer membrane from both growing and phage T4-infected *Escherichia coli* B. Biochim Biophys Acta 514:117–127

Mayrand D, Grenier D (1989) Biological activities of outer membrane vesicles. Can J Microbiol 35:607–613

Mayrand D, Holt SC (1988) Biology of asaccharolytic black-pigmented *Bacteroides species*. Microbiol Rev 52:134–152

McBroom AJ, Kuehn MJ (2007) Release of outer membrane vesicles by gram-negative bacteria is a novel envelope stress response. Mol Microbiol 63:545–558

McBroom AJ, Johnson AP, Vemulapalli S, Kuehn MJ (2006) Outer membrane vesicle production by *Escherichia coli* is independent of membrane instability. J Bacteriol 188:5385–5392

Meadow PM, Wells PL, Salkinoja-Salonen M, NE L (1978) The effect of lipopolysaccharide composition on the ultrastructure of *Pseudomonas aeruginosa*. J Gen Microbiol 105:23–28

Mug-Opstelten D, Witholt B (1978) Preferential release of new outer membrane fragments by exponentially growing *Escherichia coli*. Biochim Biophys Acta 508:287–295

Namork E, Brandtzaeg P (2002) Fatal meningococcal septicaemia with "blebbing" meningococcus. Lancet 360:1741

Nguyen TT, Saxena A, Beveridge TJ (2003) Effect of surface lipopolysaccharide on the nature of membrane vesicles liberated from the gram-negative bacterium *Pseudomonas aeruginosa*. J Electron Microsc (Tokyo) 52:465–469

Nowotny A, Behling UH, Hammond B, Lai CH, Listgarten M, Pham PH, Sanavi F (1982) Release of toxic microvesicles by *Actinobacillus actinomycetemcomitans*. Infect Immun 37:151–154

Pier GB (2000) Peptides, *Pseudomonas aeruginosa*, polysaccharides and lipopolysaccharides–players in the predicament of cystic fibrosis patients. Trends Microbiol 8:247–250; discussion 250–241

Raivio TL (2005) Envelope stress responses and gram-negative bacterial pathogenesis. Mol Microbiol 56:1119–1128

Sabra W, Lunsdorf H, Zeng AP (2003) Alterations in the formation of lipopolysaccharide and membrane vesicles on the surface of *Pseudomonas aeruginosa* PAO1 under oxygen stress conditions. Microbiology 149:2789–2795

Schnaitman CA, Klena JD (1993) Genetics of lipopolysaccharide biosynthesis in enteric bacteria. Microbiol Rev 57:655–682

Schooling SR, Beveridge TJ (2006) Membrane vesicles: an overlooked component of the matrices of biofilms. J Bacteriol 188:5945–5957

Shoberg RJ, Thomas DD (1993) Specific adherence of *Borrelia burgdorferi* extracellular vesicles to human endothelial cells in culture. Infect Immun 61:3892–3900

Smit J, Kamio Y, Nikaido H (1975) Outer membrane of *Salmonella typhimurium*: chemical analysis and freeze-fracture studies with lipopolysaccharide mutants. J Bacteriol 124:942–958

Song T, Mika F, Lindmark B, Liu Z, Schild S et al (2008) A new *Vibrio cholerae* sRNA modulates colonization and affects release of outer membrane vesicles. Mol Microbiol 70:100–111

Stephens DS, Edwards KM, Morris F, McGee ZA (1982) Pili and outer membrane appendages on *Neisseria meningitidis* in the cerebrospinal fluid of an infant. J Infect Dis 146:568

Stirling P, Richmond SJ (1980) Production of outer membrane blebs during chlamydial replication. FEMS Microbiol Lett 9:103–105

Tetz VV, Rybalchenko OV, Savkova GA (1990) Ultrastructural features of microbial colony organization. J Basic Microbiol 30:597–607

Unal CM, Schaar V, Riesbeck K (2010) Bacterial outer membrane vesicles in disease and preventive medicine. Semin Immunopathol 33:395–408

Vesy CJ, Kitchens RL, Wolfbauer G, Albers JJ, Munford RS (2000) Lipopolysaccharide-binding protein and phospholipid transfer protein release lipopolysaccharides from gram-negative bacterial membranes. Infect Immun 68:2410–2417

Wai SN, Takade A, Amako K (1995) The release of outer membrane vesicles from the strains of enterotoxigenic *Escherichia coli*. Microbiol Immunol 39:451–456

Wai SN, Lindmark B, Soderblom T, Takade A, Westermark M et al (2003) Vesicle-mediated export and assembly of pore-forming oligomers of the enterobacterial ClyA cytotoxin. Cell 115:25–35

Yonezawa H, Osaki T, Kurata S, Fukuda M, Kawakami H et al (2009) Outer membrane vesicles of *Helicobacter pylori* TK1402 are involved in biofilm formation. BMC Microbiol 9:197

Zhou L, Srisatjaluk R, Justus DE, Doyle RJ (1998) On the origin of membrane vesicles in gram-negative bacteria. FEMS Microbiol Lett 163:223–228