Editorial Commentary

Outer membrane vesicles are the powerful cell-to-cell communication vehicles that allow bacteria to monitor extracellular milieu

Meysam Sarshar[1], Daniela Scribano[2], Payam Behzadi[3], Andrea Masotti[1], Cecilia Ambrosi[4]

[1]Research Laboratories, Bambino Gesù Children's Hospital-IRCCS, Rome, Italy; [2]Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy; [3]Department of Microbiology, Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran; [4]Department of Human Sciences and Promotion of the Quality of Life, San Raffaele Open University, IRCCS, Rome, Italy

Correspondence to: Meysam Sarshar. Research Laboratories, Bambino Gesù Children's Hospital-IRCCS, Viale San Paolo, n. 15, 00146 Rome, Italy. Email: meysam.sarshar@uniroma1.it.

Comment on: Zhao Z, Wang L, Miao J, *et al.* Regulation of the formation and structure of biofilms by quorum sensing signal molecules packaged in outer membrane vesicles. Sci Total Environ 2022;806:151403.

Received: 02 August 2022; Accepted: 10 September 2022; Published: 30 November 2022.

doi: 10.21037/exrna-22-18

View this article at: https://dx.doi.org/10.21037/exrna-22-18

Existence of cell-to-cell communication strategies among bacteria (i.e., defined as inter-species, intra-species, and inter-kingdom crosstalk) reveals how genius they are (1,2). The common and well-studied language for bacterial communication is quorum sensing (QS), an informative signaling pathway within a bacterial population (3-5). Specifically, bacteria secrete signaling modules under QS regulation, known as autoinducers (AIs), to share quorum information and regulate group behaviours. QS modulates the expression of bacterial pathogenicity factors involved in the infection process, as a similar regulation could occur in the physiology of host cells. Detailed mechanisms are extensively reviewed (6,7).

Bacterial conversation enables them to feel the surrounding stimuli and coordinate their gene expression accordingly. This cooperation in sensing and responding to environmental changes could result in arrays of behaviour, ranging from symbiosis to virulence, biofilm formation, stress adaptation and natural product production, leading bacteria to live like multicellular organisms (8-10). Furthermore, bacteria need to manage competition for ecological niches and resources that favour their survival and genetic persistence. This strategy underlies mechanisms of complex interactions that occur among bacteria as well as bacteria-hosts.

While the potency of QS has gained the interest of researchers for a long time, only recently QS-regulated pathways have been reported to be involved in membrane vesicles (MVs) or outer membrane vesicles (OMVs) biogenesis in Gram-positive and Gram-negative bacteria, respectively (11). OMVs have been recognized as an alternative vehicle for cell-to-cell signaling in bacteria. This spherical membranous structure can package cargoes such as virulence factors, biologically active proteins, DNA, RNA, as well as signaling molecules, playing an important role in bacterial adaptation and survival (2,12). Despite tremendous progress in the OMVs field (e.g., involvement in disease-related processes and delivery mechanisms into host cells), there is still some uncertainty in the biogenesis of OMVs and their precise sorting mechanisms within bacterial communities.

So far, there is enough evidence connecting QS and OMVs, indicating that either QS molecules could serve as OMVs cargo or they are able to control the release of OMVs in several nosocomial pathogens (i.e., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Escherichia coli*) (13). The latter mechanism could act either by stimulating OMVs secretion, a phenomenon evidenced in *P. aeruginosa*, *Vibrio harveyi*, *Stenotrophomonas maltophilia*, as well as in plant pathogens such as *Xanthomonas oryzae* and *Xanthomonas campestris* or vice versa by inhibiting its release as reported in *Xylella fastidiosa* (14-18).

For instance, through a QS-regulated process, OMVs released by the environmental pathogen *Chromobacterium*

violaceum (*C. violaceum*) deliver the antimicrobial compound violacein to compete with other bacteria, exerting its toxicity *in vivo* even over far distances (19). As a QS metabolite produced by various bacterial species, the OMV cargo violacein contributes to a broad range of antimicrobial activities (20). Batista *et al.* showed that violacein of *C. violaceum* is not only toxic against Gram-positive bacteria, but also it induces OMV biogenesis for its own delivery and contributes to biofilm formation (19). Another example of bacterial usage of OMVs related to cope with nutritional challenges (i.e., iron-limited conditions) was reported by Lin *et al.* (21). They found that the type VI secretion system H3-dependent effector for iron uptake TseF is packed into OMVs. This finding suggested that TseF is incorporated into OMVs and interacts with the iron-binding *P. aeruginosa* quinolone signal (PQS); this interaction facilitates the delivery of the metal ion to its receptors on cell surface receptors (FptA or OprF) (21). Of note, for most bacterial pathogens, sensing, sequestering and uptaking efficiently environmental iron is critical to enable colonization and pathogenicity, and for this reason, this process involves multi-component complexes (22).

In a recent issue of *Science of The Total Environment*, Zhao *et al.* reported the isolation and purification of PQS-containing OMVs (PQS-OMVs) produced by *P. aeruginosa* and evaluated the effects of this signaling molecules on the formation and structure of *P. aeruginosa* biofilm (23). Their study indicated that *P. aeruginosa* biofilm capacity is regulated by OMV-mediated PQS that promotes the increase of bacterial biomass, leading to biofilm formation. Subsequently, the authors quantified in detail the extracellular polysaccharides (PS) and proteins, the two main matrix components of biofilms. Their analyses revealed that proteins and PS have a synergistic effect, although the expression of proteins regulated by OMV-mediated PQS played the dominant role with respect to PS in the formation of *P. aeruginosa* biofilms. Next, in a dual-species biofilm formed by *P. aeruginosa* and S. *aureus*, the research proved that OMV-mediated PQS exerted inhibitory effects on *S. aureus* growth, leading to a decrease in extracellular polymeric substances produced by *S. aureus* (23). These two major opportunistic pathogens, commonly co-isolated from cystic fibrosis patients, are often found growing together in biofilms in lungs and wounds (24). It should be noted that bacteria in diverse conditions (i.e., lab growth, hospital setting and during infection) behave differently as evidenced by phenotype changes as well as in exporting QS molecules and stimulating OMV biogenesis (25-29). For example, the traffic of PQS between the inner and outer membranes defines the yield of OMVs production. Florez *et al.* showed that OMVs production depends on PQS export rates rather than a defect in its production; therefore, the accumulation of PQS in the inner membrane resulted in a poor OMVs production due to early saturation of the export pathway (30).

Owing to the ability of OMVs to distribute widely PQS in an aqueous environment, it is reasonable to think that they can enable long distance transport of this essential element, thus allowing trans-feeding of bacteria at distant sites of the host during infection. In addition, the effective biofilm dispersion is dependent on the production of PQS-induced OMVs, which likely act as delivery vehicles for matrix-degrading enzymes. Accordingly, OMVs represent promising bacterial-derived molecules that could operate as an alternative for antibiotics for the treatment or inhibition of biofilm-forming species.

The studies connecting QS to OMVs in *P. aeruginosa* opened up new perspectives on other clinically relevant nosocomial pathogens such as multidrug-resistant (MDR) *Acinetobacter* spp (28,29). Previous studies have shown that *Acinetobacter* spp. can successfully release OMVs carrying plasmids containing resistance genes (i.e., bla_{OXA-24}) to recipient cells through horizontal gene transfer (31,32). To this point, Chatterjee *et al.* reported that *A. baumannii* strain ST 1462 releases OMVs capable to transfer an intact plasmid harbouring a carbapenem resistance gene ($bla_{\text{NDM-1}}$) during *in vitro* growth (33). Notably, these OMVs carrying $bla_{\text{NDM-1}}$ were found in an active form allowing high frequency of transformation and transmittable abilities not only intra-species (*A. baumannii*) but also inter-species (*E. coli*) (33). Carbapenem-resistant *A. baumannii*, i.e., extensively drug-resistant or pandrug-resistant isolates, are responsible for substantial life-threating hospital-acquired infections in patients with severe underlying diseases, mainly in intensive care units, often related to invasive procedures or indwelling devices (10,34,35). A recent study reported by Huang *et al.* showed an increase in the production of OMVs under antibiotic stress (36). Under stimulation by different antibiotics, *A. baumannii* releases OMVs at different levels of efficiency; compared to other tested antibiotics, levofloxacin was the strongest OMVs inducer both in particle number, protein level and particle diameter. Moreover, the stress induced by levofloxacin led to the encapsulation of large amounts of intracellular components into OMVs by activating efflux pumps proteins; AdeB, AdeA and AcrB were identified as the most

expressed proteins. Both *in vitro* and *in vivo* experiments showed that bacteria are able to pack pumped antibiotics into OMVs; this excellent drug-resistance ability becomes a strategy to kill other bacteria (36). Moreover, the orally administration of the antibiotic-loaded OMVs could kill pathogenic bacteria in the intestine as demonstrated by the effective killing of enterotoxigenic *E. coli* in a mouse model of intestinal infection (36).

More recently, Dhurve *et al.* reported that *A. baumannii* DS002 releases OMVs carrying proteins associated with cell wall/membrane biogenesis, inorganic ion transport and metabolism through QS signalling (37). The OMVs cargo content underlies the prominent role of OMVs in cell physiology, signaling activities, transport functions and pathogenesis, as well as in the defence mechanisms against host immunity (37). Interestingly, OMVs were selectively enriched in TonB-dependent transporters (TonRs), outer membrane proteins that capture and transport iron chelated by siderophores into *A. baumannii* DS002 cells. Thus, the OMV-associated TonRs appeared to play a critical role in the survival of *A. baumannii* in certain conditions such as nutrient-limiting polymicrobial environments (37). Despite only some pathogenicity properties in *A. baumannii* have been shown to be under QS control such as surface-associated motility and biofilm formation (38), the potential linkage between OMVs production mediated by QS remains to be investigated. In our opinion, OMVs biogenesis in *A. baumannii* could be induced through QS signaling and their role warrants extensive examinations.

Conclusion and future perspective

Overall, Zhao *et al.* provided new insights into the role of OMV-mediated PQS on biofilm formation, structure and composition of EPS in *P. aeruginosa* as well as inter-specific inhibitory effects of PQS in the context of microbial community. We learned that bacterial pathogens during their growth and metabolism excrete OMVs with biologically active proteins as well as transmissible DNA sequences associated with diverse functions. The multiple advantages of OMVs as drug delivery carriers, biofilm inhibitor or anti-bacteria adhesion will broaden effective/alternative therapeutic approaches. Due to the increased rates of antibiotic resistance as well as biofilm-associated infections, OMVs delivery could potentially direct us to explore innovative tools (i.e., development of artificial OMVs or the engineering of the natural ones) for clinical application in order to better control intestinal, pulmonary or even systemic infections caused by MDR pathogens. The intracellular trafficking of OMVs can be studied in detail using advanced *in vitro* models such as human tissue-derived organoids.

Acknowledgments

Funding: This research was funded by the Italian Ministry of Health (Ricerca Corrente and 5 × 1000). The research was also supported by Bandi Ateneo Sapienza, grant number RP120172B7FF9E6F to C.A. The Italian Ministry of Health and Bandi Ateneo Sapienza had no role in paper preparation and decision of publishing.

Footnote

Provenance and Peer Review: This article was commissioned by the Guest Editor (Chenyu Zhang) for the series "Extracellular RNAs and Human Health" published in *ExRNA*. The article has undergone external peer review.

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://exrna.amegroups.com/article/view/10.21037/exrna-22-18/coif). The series "Extracellular RNAs and Human Health" was commissioned by the editorial office without any funding or sponsorship. CA serves as an unpaid editorial board member of *ExRNA* from December 2021 to November 2023. This research was funded by the Italian Ministry of Health (Ricerca Corrente and 5 × 1000). The research was also supported by Bandi Ateneo Sapienza, grant number RP120172B7FF9E6F to CA. The Italian Ministry of Health and Bandi Ateneo Sapienza had no role in paper preparation and decision of publishing. Salary of MS was funded by the Italian Ministry of Health (starting grant SG-2018-12365432). This funder had no role in study design, decision to publish, and preparation of the manuscript. Salary of DS was funded by POR Lazio FSE 2014-202O and Sapienza Ateneo funding. These funders had no role in study design, decision to publish, and preparation of the manuscript. The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are

appropriately investigated and resolved.

References

1. Celluzzi A, Masotti A. How Our Other Genome Controls Our Epi-Genome. Trends Microbiol 2016;24:777-87.
2. Sarshar M, Scribano D, Ambrosi C, et al. Fecal microRNAs as Innovative Biomarkers of Intestinal Diseases and Effective Players in Host-Microbiome Interactions. Cancers (Basel) 2020;12:2174.
3. Nealson KH, Platt T, Hastings JW. Cellular control of the synthesis and activity of the bacterial luminescent system. J Bacteriol 1970;104:313-22.
4. Fuqua WC, Winans SC, Greenberg EP. Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators. J Bacteriol 1994;176:269-75.
5. Chevance FF, Hughes KT. Coordinating assembly of a bacterial macromolecular machine. Nat Rev Microbiol 2008;6:455-65.
6. Xiao Y, Zou H, Li J, et al. Impact of quorum sensing signaling molecules in gram-negative bacteria on host cells: current understanding and future perspectives. Gut Microbes 2022;14:2039048.
7. Behzadi P, Baráth Z, Gajdács M. It's Not Easy Being Green: A Narrative Review on the Microbiology, Virulence and Therapeutic Prospects of Multidrug-Resistant Pseudomonas aeruginosa. Antibiotics (Basel) 2021;10:42.
8. Tobias NJ, Brehm J, Kresovic D, et al. New Vocabulary for Bacterial Communication. Chembiochem 2020;21:759-68.
9. Pompilio A, Scribano D, Sarshar M, et al. Gram-Negative Bacteria Holding Together in a Biofilm: The Acinetobacter baumannii Way. Microorganisms 2021;9:1353.
10. Sarshar M, Behzadi P, Scribano D, et al. Acinetobacter baumannii: An Ancient Commensal with Weapons of a Pathogen. Pathogens 2021;10:387.
11. Wang M, Zhu P, Jiang J, et al. Signaling Molecules of Quorum Sensing in Bacteria. Rev Biotechnol Biochem 2020;1:002.
12. Ahmadi Badi S, Moshiri A, Fateh A, et al. Microbiota-Derived Extracellular Vesicles as New Systemic Regulators. Front Microbiol 2017;8:1610.
13. Furuyama N, Sircili MP. Outer Membrane Vesicles (OMVs) Produced by Gram-Negative Bacteria: Structure, Functions, Biogenesis, and Vaccine Application. Biomed Res Int 2021;2021:1490732.
14. Li A, Schertzer JW, Yong X. Molecular conformation affects the interaction of the Pseudomonas quinolone signal with the bacterial outer membrane. J Biol Chem 2019;294:1089-94.
15. Brameyer S, Plener L, Müller A, et al. Outer Membrane Vesicles Facilitate Trafficking of the Hydrophobic Signaling Molecule CAI-1 between Vibrio harveyi Cells. J Bacteriol 2018;200:e00740-17.
16. Bahar O, Pruitt R, Luu DD, et al. The Xanthomonas Ax21 protein is processed by the general secretory system and is secreted in association with outer membrane vesicles. PeerJ 2014;2:e242.
17. Mendes JS, Santiago AS, Toledo MA, et al. In vitro Determination of Extracellular Proteins from Xylella fastidiosa. Front Microbiol 2016;7:2090.
18. Devos S, Van Oudenhove L, Stremersch S, et al. The effect of imipenem and diffusible signaling factors on the secretion of outer membrane vesicles and associated Ax21 proteins in Stenotrophomonas maltophilia. Front Microbiol 2015;6:298.
19. Batista JH, Leal FC, Fukuda TTH, et al. Interplay between two quorum sensing-regulated pathways, violacein biosynthesis and VacJ/Yrb, dictates outer membrane vesicle biogenesis in Chromobacterium violaceum. Environ Microbiol 2020;22:2432-42.
20. Ahmed A, Ahmad A, Li R, et al. Recent Advances in Synthetic, Industrial and Biological Applications of Violacein and Its Heterologous Production. J Microbiol Biotechnol 2021;31:1465-80.
21. Lin J, Zhang W, Cheng J, et al. A Pseudomonas T6SS effector recruits PQS-containing outer membrane vesicles for iron acquisition. Nat Commun 2017;8:14888.
22. Sheldon JR, Laakso HA, Heinrichs DE. Iron Acquisition Strategies of Bacterial Pathogens. Microbiol Spectr 2016.
23. Zhao Z, Wang L, Miao J, et al. Regulation of the formation and structure of biofilms by quorum sensing signal molecules packaged in outer membrane vesicles. Sci Total Environ 2022;806:151403.
24. Yung DBY, Sircombe KJ, Pletzer D. Friends or enemies? The complicated relationship between Pseudomonas

aeruginosa and Staphylococcus aureus. Mol Microbiol 2021;116:1-15.
25. Sarshar M, Scribano D, Limongi D, et al. Adaptive strategies of uropathogenic Escherichia coli CFT073: from growth in lab media to virulence during host cell adhesion. Int Microbiol 2022;25:481-94.
26. Ambrosi C, Sarshar M, Aprea MR, et al. Colonic adenoma-associated Escherichia coli express specific phenotypes. Microbes Infect 2019;21:305-12.
27. Sarshar M, Scribano D, Marazzato M, et al. Genetic diversity, phylogroup distribution and virulence gene profile of pks positive Escherichia coli colonizing human intestinal polyps. Microb Pathog 2017;112:274-8.
28. Ambrosi C, Scribano D, Sarshar M, et al. Acinetobacter baumannii Targets Human Carcinoembryonic Antigen-Related Cell Adhesion Molecules (CEACAMs) for Invasion of Pneumocytes. mSystems 2020;5:00604-20.
29. Scribano D, Marzano V, Levi Mortera S, et al. Insights into the Periplasmic Proteins of Acinetobacter baumannii AB5075 and the Impact of Imipenem Exposure: A Proteomic Approach. Int J Mol Sci 2019;20:3451.
30. Florez C, Raab JE, Cooke AC, et al. Membrane Distribution of the Pseudomonas Quinolone Signal Modulates Outer Membrane Vesicle Production in Pseudomonas aeruginosa. mBio 2017;8:e01034-17.
31. Rumbo C, Fernández-Moreira E, Merino M, et al. Horizontal transfer of the OXA-24 carbapenemase gene via outer membrane vesicles: a new mechanism of dissemination of carbapenem resistance genes in Acinetobacter baumannii. Antimicrob Agents Chemother 2011;55:3084-90.
32. Fulsundar S, Harms K, Flaten GE, et al. Gene transfer potential of outer membrane vesicles of Acinetobacter baylyi and effects of stress on vesiculation. Appl Environ Microbiol 2014;80:3469-83.
33. Chatterjee S, Mondal A, Mitra S, et al. Acinetobacter baumannii transfers the blaNDM-1 gene via outer membrane vesicles. J Antimicrob Chemother 2017;72:2201-7.
34. Isler B, Doi Y, Bonomo RA, et al. New Treatment Options against Carbapenem-Resistant Acinetobacter baumannii Infections. Antimicrob Agents Chemother 2019;63:e01110-18.
35. Bartal C, Rolston KVI, Nesher L. Carbapenem-resistant Acinetobacter baumannii: Colonization, Infection and Current Treatment Options. Infect Dis Ther 2022;11:683-94.
36. Huang W, Zhang Q, Li W, et al. Development of novel nanoantibiotics using an outer membrane vesicle-based drug efflux mechanism. J Control Release 2020;317:1-22.
37. Dhurve G, Madikonda AK, Jagannadham MV, et al. Outer Membrane Vesicles of Acinetobacter baumannii DS002 Are Selectively Enriched with TonB-Dependent Transporters and Play a Key Role in Iron Acquisition. Microbiol Spectr 2022;10:e0029322.
38. Mayer C, Muras A, Parga A, et al. Quorum Sensing as a Target for Controlling Surface Associated Motility and Biofilm Formation in Acinetobacter baumannii ATCC® 17978TM. Front Microbiol 2020;11:565548.

doi: 10.21037/exrna-22-18
Cite this article as: Sarshar M, Scribano D, Behzadi P, Masotti A, Ambrosi C. Outer membrane vesicles are the powerful cell-to-cell communication vehicles that allow bacteria to monitor extracellular milieu. ExRNA 2022;4:25.