Journal of Global Antimicrobial Resistance 32 (2023) 104–107

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Short Communication

Detection of *Morganella morganii* bound to a plastic substrate in surface water

Ifra Ferheen[a], Roberto Spurio[a], Laura Mancini[b], Stefania Marcheggiani[b,*]

[a] School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy
[b] Department of Environment and Health, Italian National Institute of Health, Rome, Italy

ARTICLE INFO

Article history:
Received 12 October 2022
Revised 29 December 2022
Accepted 26 January 2023
Available online 9 February 2023

Editor: Dr Jon Hobman

Keywords:
Morganella morganii
Artificial plastic substrate
Aquatic ecosystem
One-Health
Antibiotic resistance genes

ABSTRACT

Objectives: Around the globe, escalation in rare opportunistic microbial infections is alarming as they are heading steadily towards 'superbug' status. In aquatic ecosystems, plastic fosters multidrug-resistant pathogenic bacteria and plays a significant role in trafficking antibiotic-resistant genes. In this study, we focused on a multidrug-resistant bacterial strain isolated from microbial communities found on plastic substrates of a volcanic lake in central Italy.

Methods: Extended-spectrum beta-lactamase-producing strains were isolated from both raw water and plastic substrates for a comparative investigation using microbiological and molecular methods, and antibiotic susceptibility profiling was performed against a panel of ten antibiotics.

Results: Molecular identification and Basic Local Alignment Search Tool analysis confirmed an almost identical sequencing pattern of two isolated strains and their homology with *Morganella morganii*. Antibiotic susceptibility tests revealed their resistance to almost all tested antibiotics. Class 1 integron-associated gene (intI1) and seven antibiotic resistance genes were detected in both strains, confirming their superbug status.

Conclusion: To our knowledge, this is the first study on the characterization of extended-spectrum beta-lactamase-producing *M. morganii* isolated from the biofilm of plastic substrates, depicting the potential toxicity of plastic in harbouring and dispersing virulent, multidrug-resistant, opportunistic human pathogens.

1. Introduction

In the golden era of antibiotics, humans tipped the scales in their favour; however, this dependency on antibiotics has become alarming in the post-antibiotic era [1]. The scientific community is worried as various unusual and opportunistic microorganisms are smartly climbing the evolutionary ladder without any significant recognition from concerned authorities [2]. Water-related pathogens are considered one of the crucial concerns and challenges to public health across the globe [3].

Morganella morganii is a Gram-negative rod-shaped, opportunistic bacterium predominantly causing nosocomial infections or neonatal sepsis [4]. It is found in the environment and in the intestinal tract of humans, mammals, and reptiles as part of the normal flora. The vast host range and atypical symbiotic relationships established by *M. morganii* are also rising threats as they could result in even more complex infections [5]. Traditionally, the pathogenicity of *M. morganii* has been exclusively associated with summer diarrhea; however, in the past few years, almost every continent has been reporting its increased detection in all age groups, causing severe organ damages like brain and liver abscess, chorioamnionitis, septic arthritis, chronic osteomyelitis, and rhabdomyolysis [2].

Horizontal gene transfer of antibiotic-resistant genes (ARGs), often mediated by plasmids among strains of the Enterobacteriaceae family, including *M. morganii*, is a primary health concern, as it leads to increased morbidity and mortality rates by evading all available treatments. The spread of *M. morganii* and other multidrug-resistant (MDR) pathogens into aquatic ecosystems is overlooked as it is not a mandatory parameter for assessing water quality in monitoring programs [6]. From the One-Health

* Corresponding author. Mailing address: Department of Environment and Primary Prevention, National Institute of Health, Viale Regina Elena, 299, 00161 Rome, Italy
E-mail address: stefania.marcheggiani@iss.it (S. Marcheggiani).

https://doi.org/10.1016/j.jgar.2023.01.008

perspective, the aquatic environment could be a potential reservoir for such pathogenic bacteria. In aquatic ecosystems, plastics are emerging pollutants that act as a trap for amplifying pathogens by forming sturdy biofilms, playing a vital role in trafficking MDR bacteria and ARGs [7]. Plastics colonized by potentially virulent animal and human pathogens may also affect aquaculture. Even though this issue has drawn considerable attention from many researchers, very scarce information is available on their role as the vector for MDR bacteria and ARGs [8].

Considering complex interactions, symbiotic behavior, rising resistance, and infection rates of *M. morganii*, its colonization on the plastic surface should be strictly monitored to address public concerns. Hence, in-depth follow-up studies are needed to understand the role of plastic in spreading MDR *M. morganii* in the aquatic environment. The current research focuses on detecting extended-spectrum beta-lactamase- (ESBL) producing *M. morganii* on plastic surfaces submerged in a lake in central Italy as an artificial substrate.

2. Materials and methods

In the late summer of 2021, Artificial Plastic Substrates (APSs) were submerged deep (1.5 meters) in the euphotic zone at seven geographical sites of the volcanic lake of Bracciano (Italy). The APSs were kept in the lake for one month to allow the colonization of pathogens. A comparative analysis of the microbial community found in water and in APS was performed to investigate plastic's potential role in amplifying and spreading pathogenic bacteria. Upon collection of the APSs and raw water, samples were transported to the laboratory under sterile and dark conditions at 4°C, and further analyses were performed within 24 h. APSs with the grown plastisphere were repeatedly treated with sterile scrapers and swabs; the collected material was inoculated into pre-enrichment Muller-Hinton (MH) broth and incubated at 37°C for 24 h (Kangda Intercontinental Medical Equipment, Zhejiang Province, China). Two L of raw water samples collected from each site were filtered using a cellulose nitrate filter with a 0.45 µm diameter pore size (Sartorius Stadium Biotech, Goettingen, Germany), and the filters were incubated for pre-enrichment into MH broth, as described above. Aliquots of 100 µL of pre-enriched culture were spread onto a chromogenic medium for ESBL screening (Chrom-ESBL agar, Biomerieux, Marcy-l'Étoile, France) and incubated at 30°C for 24 h. Single colonies were isolated onto MH agar plates at 37°C for 24 h.

For species identification, morphologically homogeneous colonies were used to extract genomic material using a DNA extraction kit (GRiSP, Porto, Portugal). Extracted DNA was amplified by 16S rDNA gene universal primers F (5′-AGAGTTTGATCMTGGCTCAG-3′) and R (5′- TACGGYTACCTTGTTACGACTT -3′). Purified polymerase chain reaction products were sequenced (Eurofins, Germany), and the results were analysed by Basic Local Alignment Search Tool analysis and MEGA11 (Molecular Evolutionary Genetic Analysis software).

Antibiotic susceptibility tests were performed using the Kirby-Bauer disk diffusion assay and interpreted as per Clinical Laboratory Standard Institute guidelines [9]. The isolated strains were evaluated against ten commercially available antibiotics (Oxoid, Basingstoke, Hampshire, UK): tetracycline (TET 30 µg), gentamycin (GEN 10 µg), chloramphenicol (CAM 30 µg), ciprofloxacin (CIP 1 µg), meropenem (MEM 10 µg), imipenem (IMP 10 µg), kanamycin (KAN 30 µg), cefoxitin (FOX 30 µg), sulfamethoxazole (SXM 25 µg), and ceftazidime (CAZ 30 µg). *Klebsiella pneumoniae* ATCC13883 strain was used as a positive control for an antibiotic susceptibility quality check.

Ten ARGs belonging to four different antibiotic classes; tetracycline (tetA, tetB, tetC), sulfonamide (sul1, sul2, sul3), chloramphenicol (cmlA1, cmx(A)), β-lactamase (blaCTX-M, blaTEM) and class 1 integrons (intI1 and intI1-V) were investigated by molecular tools. Genomic DNA isolated from the two strains under investigation was used as a template for polymerase chain reaction amplification using primers and conditions previously described [10].

3. Results and discussion

Detection and adverse effects of plastic debris in organisms living in the marine environment are corroborated by a considerable amount of scientific data reported in the literature; however, almost no description is currently available on the occurrence of this phenomenon in inland waters, especially in terms of their ability to disperse resistant pathogenic bacteria [11]. The lake investigated in this study is one of central Italy's most important surface drinking water reservoirs and a tourist attraction spot during the summer season [12]. An efficient sampling protocol was designed and optimised to achieve efficient and reproducible isolation of plastic-colonizing bacteria, which were analysed in a side-by-side comparison with planktonic bacteria found in raw water. Chromogenic ESBL agar medium is a selective medium for screening broad-spectrum β-lactamase-producing Enterobacteria. When observed under a microscope, colonies obtained from plastic artificial substrate grown on ESBL agar displayed light brown to dark brown phenotype. Bacterial colonies showing the same phenotype were present on the APS of two of the seven geographical sites. However, ESBL-producing bacteria isolated from raw water samples collected from all lake sites did not display such phenotype.

Molecular identification and Basic Local Alignment Search Tool analysis of brownish colonies confirmed the almost identical sequencing pattern of two isolated strains. The 16S rDNA sequence of two isolated strains, which revealed 99% of identity with strains of *M. morganii* available in the database, were submitted to the National Center for Biotechnology Information Database and assigned accession numbers OP420858 and OP420859. The Kimura two-parameter model (K2P) was used to determine the model of nucleotide substitution that best fit the data. Evolutionary analyses were conducted using the MEGA11 software. The maximum parsimony tree was drawn to scale, with branch lengths measured as the number of substitutions per site. The phylogenetic analysis of obtained rDNA sequences illustrated a close relationship with clinical and environmental strains; in particular, OP420858 with two clinical isolates of *M. morganii* (HQ169114.1 and MN7446971) and OP420859 with strain MG654671.1 isolated from the fish Micropterus salmoides (Fig. 1).

The antibiotic susceptibility test results against the panel of antibiotics commonly used in animal and human therapeutics (Table 1) demonstrated that both benthic *M. morganii* strains were resistant to all tested antibiotics except imipenem.

More detailed information about the antibiotic resistance phenotypic profile of the two strains was obtained by applying genotypic characterization of ARGs. Molecular detection of ARGs and class 1 integron in both plastic-associated *M. morganii* strains revealed their remarkable resistance pattern (Table 2). Surprisingly these isolates were carrying several clinically significant ARGs, particularly one tetracycline resistance gene (tetC), two sulfonamide resistance genes (sul1, sul3), two chloramphenicol genes (cmlA1, cmx(A)) and two β-lactamase resistance genes (blaCTX-M-01, blaCTX-M-02). In addition, one gene coding for class 1 integron (intl1), which is involved in the dissemination of ARGs, was detected.

A similar resistance pattern against commonly used antibiotics and detection of ARGs in pathogenic bacteria, including the *M. morganii* strain isolated from marine plastic debris, has been recently reported in the literature [13,14]. Based on the detection of MDR human pathogens carrying several ARGs on a plastic sub-

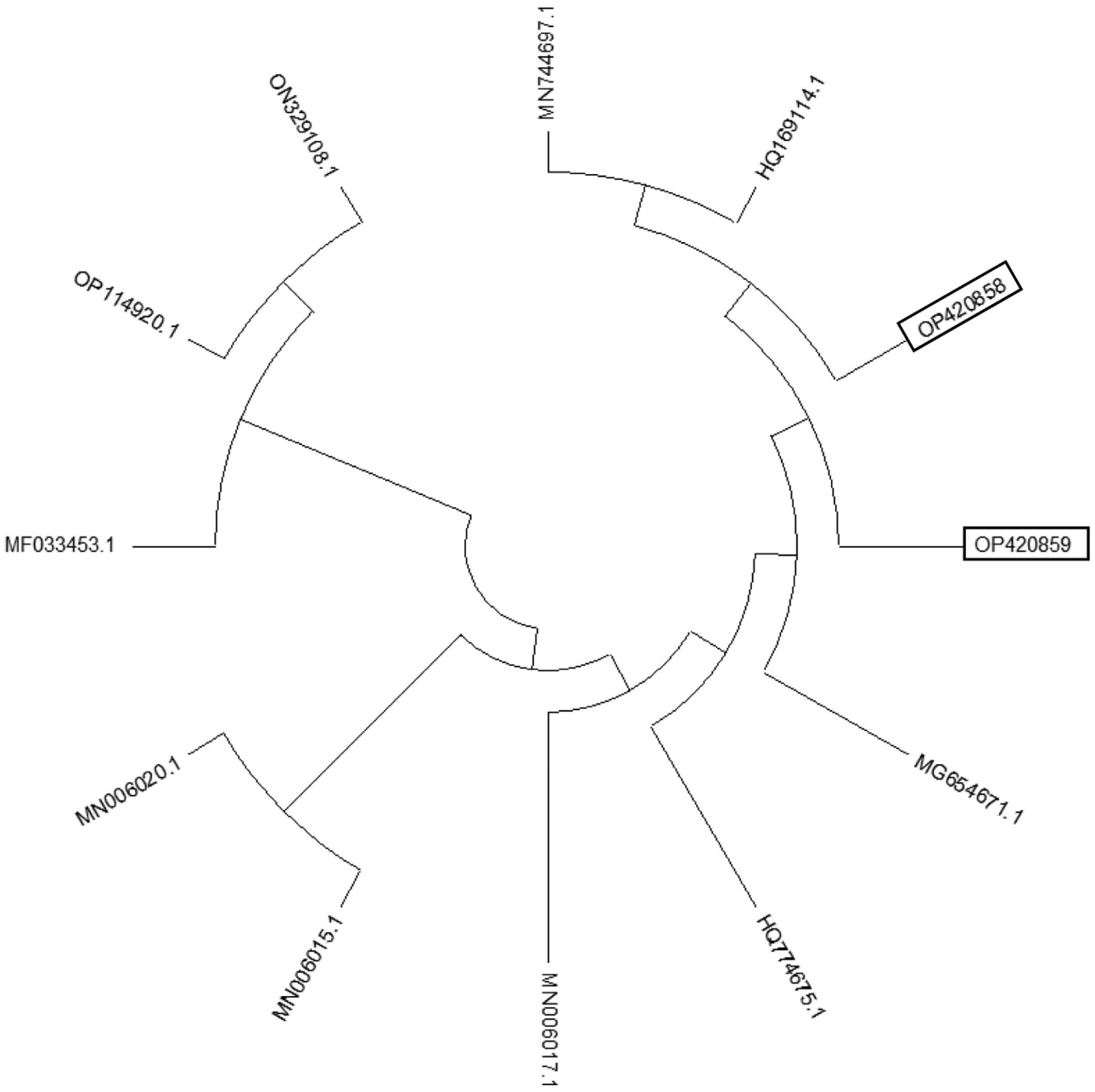

Fig. 1. Phylogenetic tree of the DNA sequences obtained from APS adhering bacteria, aligned with known strains of *Morganella morganii*. The two sequences of pathogenic strain obtained from APSs are shown in the rectangle box.

Table 1
Antibiotic susceptibility profile of two pathogenic strains of *M. morganii*.

Accession number	Tested Antibiotics									
	TET	GEN	CAM	FOX	CIP	IMP	SXM	KAN	CAZ	MEM
M. morganii OP420858	R	R	R	R	R	I	R	R	R	R
M. morganii OP420859	R	R	R	R	R	I	R	R	R	R

TET, tetracycline; GEN, gentamycin; CAM, chloramphenicol; FOX, cefoxitin; CIP, ciprofloxacin; IMP, imipenem; SXM, sulfmethoxazole; KAN, kanamycin; CAZ, ceftazidime; MEM, meropenem; R, Resistant; I, Intermediate [9].

Table 2
Molecular detection of antibiotic resistance genes in isolated *M. morganii* strains.

Accession number	Antibiotic Resistance Genes										Class 1 Integron	
	cmlA1	cmx(A)	sul1	sul2	sul3	tetA	tetB	tetC	blaCTX-M-01	blaCTX-M-02	intI1	intI1-V
M. morganii OP420858	+	+	+	-	+	-	-	+	+	+	+	-
M. morganii OP420859	+	+	+	-	+	-	-	+	+	+	+	-

strate, it could be speculated that microplastics are durable islands, providing hotspots for their colonization, evolution, and dissemination. MDR and extensively drug-resistant clinical strains of *M. morganii* are frequently reported around the globe [4], as this microorganism now possesses resistance to first-, second-, and third-generation cephalosporins, carbapenems, lincosamides, macrolides, and beta-lactam antibiotics.

The detectable presence of extensively resistant *M. morganii* strains exclusively on the artificial substrate may depict the potential role of plastic in amplifying the pathogenicity of bacteria. Plastics provide a hydrophobic surface for the attachment of microorganisms, promoting colonization and biofilm formation. Under this condition, commonly found on medical implant surfaces, bacteria form a complex and multi-layered three-dimensional architecture, which offers protection from a wide range of environmental challenges and favours the exchange of drug-resistance genes [15]. *M. morganii* has developed a remarkable ability to adhere to different surfaces, form biofilms, and acquire highly efficient MDR. In the clinical sector, the biofilm-forming potential of such pathogenic bacteria is a significant problem, which is well documented and refers to hospital-acquired infections transmitted by catheters [16]. Our findings provide initial information on the role of the aquatic ecosystem in spreading superbugs attached to the surface of artificial plastic substrates, suggesting their role as a 'vector' for the spread of antibiotic resistance and the diffusion of pathogens such as *M. morganii*.

4. Conclusion

The environmental fate of plastic debris and its role in trafficking pathogenic microorganisms and antibiotic resistance genes to humans and animals is still an open debate topic in the literature, with only weak and scant scientific evidence reported from the One-Health perspective. In a nutshell, our study strengthens the notion that plastic debris in an aquatic ecosystem is an emerging hazardous pollutant, boosting the colonization of superbugs and aiding the transfer of ARGs. This phenomenon can enhance their dispersal and transmission to humans and animals. Consequently, direct and indirect contact with this contaminated debris could be one potential but underestimated route of exposure to such lethal pathogenic bacteria. The significance of this information is not limited to the realm of public health but also relevant to concerned authorities who must enact the necessary measures to manage both plastic pollution and antibiotic misuse.

Funding

No funding was obtained for this study.

Ethical Approval

No ethical approval was required for this study as no human or animal models were involved.

Competing Interests

The authors have no conflicts of interest to declare.

References

[1] de Mol ML, Snoeck N, de Maeseneire SL, Soetaert WK. Hidden antibiotics: where to uncover? Biotechnol Adv 2018;36:2201–18. doi:10.1016/j.biotechadv.2018.10.008.
[2] Bandy A. Ringing bells: *Morganella morganii* fights for recognition. Public Health 2020;182:45–50. doi:10.1016/J.PUHE.2020.01.016.
[3] Ahmed T, Scholz M, Al-Faraj F, Niaz W. Water-related impacts of climate change on agriculture and subsequently on public health: a review for generalists with particular reference to Pakistan. Int J Environ Res Public Health 2016;13:1051. doi:10.3390/ijerph13111051.
[4] González LV, Rezaei N. Human pathogenic *Enterobacterales*. In: Encyclopedia of Infection and Immunity. Elsevier; 2022. p. 628–36.
[5] Minnullina L, Pudova D, Shagimardanova E, Shigapova L, Sharipova M, Mardanova A. Comparative genome analysis of uropathogenic *Morganella morganii* strains. Front Cell Infect Microbiol 2019;9:167. doi:10.3389/FCIMB.2019.00167/BIBTEX.
[6] Dettori M, Arghittu A, Deiana G, Castiglia P, Azara A. The revised European Directive 2020/2184 on the quality of water intended for human consumption. A step forward in risk assessment, consumer safety and informative communication. Environ Res 2022;209:112773. doi:10.1016/J.ENVRES.2022.112773.
[7] Arias-Andres M, Klümper U, Rojas-Jimenez K, Grossart HP. Microplastic pollution increases gene exchange in aquatic ecosystems. Environ Pollut 2018;237:253–61. doi:10.1016/J.ENVPOL.2018.02.058.
[8] Can-Güven E. Microplastics as emerging atmospheric pollutants: a review and bibliometric analysis. Air Qual Atmos Health 2020;14:203–15. doi:10.1007/s11869-020-00926-3.
[9] Humphries R, Bobenchik AM, Hindler JA, Schuetz AN. Overview of changes to the Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing, M100, 31st Edition. J Clin Microbiol 2021;59:e0021321. doi:10.1128/JCM.00213-21.
[10] Zhang Y, Lu J, Wu J, Wang J, Luo Y. Potential risks of microplastics combined with superbugs: enrichment of antibiotic resistant bacteria on the surface of microplastics in mariculture system. Ecotoxicol Environ Saf 2020;187:109852. doi:10.1016/j.ecoenv.2019.109852.
[11] Rezania S, Park J, Md Din MF, Mat Taib S, Talaiekhozani A, Kumar Yadav K, et al. Microplastics pollution in different aquatic environments and biota: A review of recent studies. Mar Pollut Bull 2018;133:191–208. doi:10.1016/j.marpolbul.2018.05.022.
[12] Rossi D, Romano E, Guyennon N, Rainaldi M, Ghergo S, Mecali A, et al. The present state of Lake Bracciano: hope and despair. Rendiconti Lincei 2019;30:83–91. doi:10.1007/s12210-018-0733-4.
[13] Silva MM, Maldonado GC, Castro RO, de Sá Felizardo J, Cardoso RP, Dos Anjos RM, et al. Dispersal of potentially pathogenic bacteria by plastic debris in Guanabara Bay, RJ, Brazil. Mar Pollut Bull 2019;141:561–8. doi:10.1016/j.marpolbul.2019.02.064.
[14] Radisic V, Nimje PS, Bienfait AM, Marathe NP. Marine plastics from Norwegian west coast carry potentially virulent fish pathogens and opportunistic human pathogens harboring new variants of antibiotic resistance genes. Microorganisms 2020;8:1200. doi:10.3390/microorganisms8081200.
[15] Wu X, Pan J, Li M, Li Y, Bartlam M, Wang Y. Selective enrichment of bacterial pathogens by microplastic biofilm. Water Res 2019;165:114979. doi:10.1016/j.watres.2019.114979.
[16] Learman BS, Brauer AL, Eaton KA, Armbruster CE. A rare opportunist, *Morganella morganii*, decreases severity of polymicrobial catheter-associated urinary tract infection. Infect Immun 2019;88 e00691–19. doi:10.1128/IAI.00691-19.