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Antibiotic Resistance Bacteria from Rivers Water in Algeria

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Abstract

The aim of the current investigation was to look on the presence of the antibiotic resistance bacteria in natural environment in Algeria. From fresh water, multidrug resistant bacteria were harvested, and then investigated for their resistance profile and extend spectrum Beta lactamase and metallo Beta lactamase production. From isolate bacteria only *Aeromonas hydrophila* catch our attention, because of their metallo Beta lactamase production and alimentary and clinical impact. This finding confirmed our hypothesis that natural environment could be colonized by multidrug resistant bacteria especially to carbapenem antibiotic who may be induced by mutation or horizontal gene transfer. The spread of these kinds of resistance organisms may become a serious issue for the public health and food security.

Keywords: *Aeromonas hydrophila*; Antibiotic resistance; River; Algeria

Introduction

Freshwater constitute favourable living ecosystem for numerous organisms such as *Aeromonas* spp [1]. Water constitutes not only a way of dissemination of antibiotic-resistant organisms among human and animal population, as drinking water is produced from surface water, but also the route by which resistance genes are introduced in natural bacterial ecosystems. Many of these bacteria harbour antibiotic resistance genes, mainly inserted in plasmid, transposant or intergron able to be transferred among water and soil bacteria community via horizontal gene transfer [2].

Aeromonas are Gram negative bacilli organisms. They occupied several environmental areas including: aquatic habitats, fish, foods, domesticated pets, invertebrate species, birds, ticks and insects and natural soils [3]. *Aeromonas* are involved in several human infections, mainly in gastroenteritis, cellulites, meningitis, bacteraemia, soft tissue infections, peritonitis and brochopulmonary infection [4]. *Aeromonas* genome species (*A. hydrophila*, *A. caviae* and *A. veronni* bv *sorbia*) are the main actor for the majority of human infections [5].

Against these infections; therapeutic molecule failed because of the resistance mechanism evolved by pathogenic agent. Antibiotic resistance is one of the major clinical issues. As predicted by Fleming the microbial organisms would evolve resistance mechanisms against the action of the antibiotics [6]. In fact, numerous resistance mechanisms have been reported since Fleming predicted this phenomenon. One of the most common mechanisms is against β lactam antibiotics, by production of β lactamases. Within *Aeromonas* genus, Beta lactam resistance principally mediated by three classes of β lactamases including, class C cephalosporinase, class D penicillinase and class B metallo Beta Lactamase [5].

Due to the overuse of broad spectrum antibiotics in clinical settings, agriculture, and fish hatcheries, antibiotic resistance has dramatically raised among disease causing *Aeromonas* [7].

There by, *Aeromonas* species received an increased attention from clinical investigators, because of their implication in serious human infections and antibiotic resistant rise within clinical [8].

Outside of the clinical environment, *Aeromonas* species express shy multidrug resistance. From aquatic samples, Aeromonas species have been reported to be susceptible to a variety of antimicrobial agents [9]

The current study was designed to evaluate the antibiotic resistance of bacilli Gram negative isolated from freshwater in Algeria. The investigations lunched with hypothesis that natural environment could be colonized by multidrug resistant bacteria especially to carbapenem antibiotic.

Materials and Methods

Sampling

Current study investigated on multidrug resistant Gram negatives bacteria. Around 15 samples were harvested from water river, sludge and wastewater in Setif region "Ait ourthilane" (Algeria) during winter period (2015).

Bacterial isolation and identification

From each sample serial dilution were prepared then enriched. One hundred micro litres from enrichment bacterial suspension was streaked on Mac Conkey (Himedia, USA) and Chromocult medium (Oxoid, UK) both supplemented with imipenem (0.5 µl/mg) and vancomycin (16 µg/ml). Then, isolates were purified on Chromocult medium (Oxoid, UK) with imipenem (0.5 µl/mg) and conserved in agar slants [10].

The identification of isolates was carried out using API 20E (BIOMERIEUX, France). The obtained results were analysed using apiWeb BIOMERIEUX resources (https://apiweb.biomerieux.com/servlet/Authenticate).

Antimicrobial agent and resistance study

For this study we used the following antimicrobial agent: cefotaxim (CTX 30µg), ceftazidime (CAZ 30µg), aztreonam (ATM 30µg), ertapenem (ETP 10µg), meropenem (MEM10µg), imipenem (IMP 10µg) and amikacin (AK 30µg) (Oxoid, UK). The susceptibility and resistance of the isolates was performed following the disc diffusion method. Overnight culture resuspended in 0.9% of saline solution. Bacterial suspension with turbidity equivalent to 0.5 Mac Farland were inoculated by streaking the cotton swab on Muller Hinton agar (Oxoid, UK) plates then incubated at 37°C. After 24 h, the inhibition zones were measured and compared to guide line of the EUCAST (2014) [11].

From the isolates, multidrug resistant strains selected for the detection of extend spectrum beta lactamase (ESBLs) and metalo β lactamase (MBL) production. ESBL production was performed with double disc synergy test essay (DDST) [12]. Then revelation of the metalo β lactamase (MBL) carried out following the Ethylene diamine tetra acetic acid (EDTA), combined disc [13] and synergy test [14]. Then Carba NP [15] and Hodge test [12] were carried out.

Results

In our investigation, from water and sludge river in Setif region "Ait Ourthilane" 30 isolates were obtained and only one strains from river water *Aeromonas hydrophila* RWBOCw3 with API 20 E profile: 1247521, showed interesting resistant profile against carbapenem antibiotic, especially imipenem, ertapenem cefotaxim and ceftazidime, while *Aeromonas hydrophila* RWBOCw3 were sensitive to meropenem, amikacin and Aztreonam Table 1. First investigation on the detection of ESBLs (DD test) did not show any formation of ghost inhibition zones between the central discs suggesting that carbapenem resistance could not be mediated by production of ESBL. While production of MBL was confirmed by positive results from Hodge and EDTA test; an distorted inhibition zone was observed on Hodge test plates and synergy image was formed and difference was observed between the inhibition zone of imipenem+EDTA and the inhibition zone of imipenem alone. With Carba NP test, red methyl changed the colour to yellow within 20min after incubation indicating possible carbapenemase carriage.

Antibiotic	IMP (10 µg)	MEM (10 µg)	ETP (10 µg)	CAZ (30 µg)	CTX (30 µg)	AK (30 µg)	ATM (30 µg)	ESBL	MBL
Diameter zone (mm)	22	25	15	6	15	26	40	None	Yes

Table 1: Resistance profile of *Aeromonas hydrophila* RWBOCw3 isolate.

Discussion

Outside of the clinical environment, antibiotic molecules may be detected in natural environment. The antibiotic molecules are naturally produced by environmental micorbiota, though in concentration much lower compared to those used in therapy [16]. From nature, most potent molecule occurring β lactam were carbapenems and olixanic acids [17]. The natural environment may be the most important source of antibiotic resistance genes Czekalski et al. [18], reported that freshwater aquatic environment represent a potential reservoir of antibiotic genes such as sulfamides, tetracycline and fluroquinolone resistant genes [19].

From Seine River, France, Girlich and co-workers confirmed theirs hypothesis that *Aeromonas* spp could be an important environmental reservoir for ESBLs; a variety of ESBLs genes were detected from *Aeromonas* spp resistant to ceftazidime [9]. Other investigation from Eastern Basin of Lake Erie in Pennsylvania, *Aeromonas* spp (*A. hydrophila* and *A. veronii*) express resistant to tetracycline and ciprofloxacin [7].

Collection of water samples from different aquatic environments within an urban water cycle in the region of Northern Portugal followed by resistance profile determination reveal resistance to ceftazidime or meropenem in isolates from the drinking water treatment plant and waste water isolates were intrinsically resistant to nalidixic acid [20].

Within *Aeromonas* species, resistance to cephamycins and extend spectrum cephalosporins mediated by class C cephalosporinase (AmpC family). While, class B Metallo Beta Lactamase induced by CphA type within *Aeromonas hydrophila* and *Aeromonas veronii* clinical and environmental isolates express resistance to ampicillin, oxacillin, cephaloridine and imipenem [21]. Two other metallo beta lactamase (MBLs) (VIM and IMP) have identified in strains of *Aeromonas hydrophila* and *Aeromonas caviae*, harboured on an integron and a plasmid, respectively [22,23].

Besides, misuse of antibiotic in clinical and veterinary set, anthropogenic human activity, spontaneous mutation and horizontal gene transfer are the principal actor in the dissemination and persistence of multidrug resistance in environment [24]. Resistant genes carried on mobile element such plasmid, integron and transposon led to spread among bacterial environment, even in the presence of selective pressure or antibiotic inducer at high or low concentration [9].

Depending on the bacterial population hosted in the environment, whether pathogens or no pathogens, the environment has an important influence on the development of multidrug resistant. Driven on chromosome or extra-chromosome or associated with mobile genetic element such as plasmids, integrons, genes cassettes or transposons, resistant genes are horizontal and vertical transferred from resistance to sensitive strains [25] causing the dissemination of antibiotic resistance within bacterial pathogen natural and hospital environment [19]. Spread of that kind of strains may create serious therapeutic problems in the future [26].

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