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ANTIMICROBIAL RESISTANCE PROFILE STUDY OF CADMIUM- RESISTANT BACTERIA

LILA YAKOUBI [1] & BASSEM JAOUADI [2]

[1]Laboratory of Cellular and Molecular Biology, Faculty of Biological Sciences,
University of Sciences and Technology of Houari Boumediene (USTHB), Bab Ezzouar, Algeria

[2] Laboratory of Microbial Biotechnology and Enzyme Engineering (LBMIE)
Sfax Biotechnology Center (CBS), University of Sfax, Tunisia

ABSTRACT

Antibiotic resistance is now reaching dangerously high levels in all regions of the world. New resistance mechanisms are emerging and spreading around the world, compromising the ability to treat common infectious diseases. For an increasing number of infections, treatment becomes more difficult, sometimes impossible, due to the loss of effectiveness of antibiotics.

This study was carried out to assess the antibiotic resistance profiles of 12 cadmium-resistant bacteria isolated from polluted cement plant soil in Algeria.

The isolates showed high resistance to cadmium was differentiated into eight genera. *Aeromonas* was the predominant (4 isolates). Followed by *Pseudomonas* (2), the others were *Burkholderia* (1), *Pasteurella* (1) *Salmonella* (1), *Shebelle* (1), *Bacillus* (1) and *Raoultella* (1).

The strains were subjected to eleven antibiotics families from the clinical panel, including Aminopenicillins, Monobactams Amino sides Fluoroquinolones Glycopeptides – Carbapenems, Cephalosporins, pencils, Rifamycins, Tetracycline, Polymyxins,

Sulfamides-Trimethoprim. The majority of the characterized germs are known to cause nosocomial infections.

Results showed that all bacterial strains were resistant, at least to three different families of antibiotics tested, they are therefore considered as multiresistant.

The findings of this study will provide a help design improved polluted soils treatment strategies to contain the spread of drug resistance which poses a great public health risk.

KEYWORDS: Bacteria, Environment, Pollution, Public Health, Resistant To Antibiotics, Resistance to Cadmium

INTRODUCTION

Nowadays, an increasing number of bacterial species known for their ubiquitous distribution in the environment are involved in nosocomial infections (acquired in hospitals) and include the list of opportunistic pathogens (Martinez and Boquerón, 2002). These bacteria are particularly difficult to eradicate because they frequently exhibit multi-resistance to antibiotics.

Antibiotics are extensively used in human, veterinary and agriculture and considered as the most important factor promoting the emergence, selection and dissemination of resistant organisms (Drought on, 2002).

This awareness has led to a more moderate and adequate use of antibiotics in clinical settings, which seems to have only a moderate effect on reducing the frequency of isolation of these bacteria (Cook et al., 2004)

It is becoming increasingly clear that the presence of other compounds causing stress conditions for indigenous bacterial communities would be implicated in the acquisition of mechanisms of resistance to antibiotics in the environment. In particular, studies suggest that contamination by heavy metals with natural, man-made or human-made ecosystems would represent an important evolutionary force for the selection of these resistances.

The toxicity of metals to organisms can lead to pollution of ecosystems, structural and functional changes in the communities present. These Changes arise from the implementation of adaptation mechanisms either the scale of the cells (gene expression, enzymatic activity, etc.) or at the scale of the populations (gene transfers). Thus, various studies have been able to highlight, in soil, sediment or aquatic environments contaminated with heavy metals, a higher proportion of resistant bacteria (Roane and Kellogg, 1996, Nazareth, et al. 2003).

On the other hand, different studies show a positive correlation between the presence of metals in an ecosystem and the resistance to both these metals and antibiotics in native bacteria, independently of metal or antibiotic (Baker-Austin, et al,.2006).

Heavy metals, recently prequalified with metallic trace elements, are present Naturally in the trace environment and their concentration can be increased by various human activities (industrial, agricultural, mining). Some of these elements such as zinc, copper or iron is essential to life at low doses and become toxic at high concentrations. Others have high toxicity even at low dose, such as mercury or cadmium.

The aim of this study is to determine the antimicrobial resistance profile of highly twelve cadmium- resistant bacteria isolated from polluted cement plant soil in Algeria.

MATERIALS AND METHODS

Determination of Antibiotic Sensitivity

As heavy metal-resistance is linked with antibiotic-resistant, the isolates were tested for their resistance to seventeen antibiotics using Mueller-Hinton agar and the disc diffusion method. Standard antibiotic-impregnated discs were placed on freshly prepared lawns of each isolate on Mueller-Hinton plates.

After 24 h to 48 h of incubation at 30°C. The diameters of the zones of inhibition were measured to the nearest millimeter for each of the antibiotics tested. The antibiotic breakpoints were interpreted using standard recommendations of the National Committee for Clinical Laboratory Standards (NCCLS, 2000).Control plates were incubated without the antibiotic discs.

The table 1 shows the families of antibiotics tested a with their main molecules as well as the disks charge

Table 1: Tested Antibiotics (Molecules, Codes and Disk Charge)

Antibiotic Family	Molecules	Code	Disk Charge (µg Or UI)
Aminopenicillins	Ampicillin	AM	10 25
Monobactams	Aztreonam	ATM	30
Aminosides	TobramycinGentamycin Kanamycin	TOB GEN K	10 10 30
Fluor quinolones	Ciprofloxacin Nalidixic	CIP NA	5 30
Glycopeptides Carbapénémes	Vancomycin Imipenem	V IPM	30 10
Cephalosporins	Ceftazidine Cefotaxime	CAZ CTX	30 10
Phenicols	Chloramphenicol	C	30
Rifamycins	Rifampicin	RA	5
Tetracyclines	Tetracycline	TE	30
Polymyxins	Colistin sulfate	CS	10
Not classified	Novobiocin	NV	30
Sulfamides-Trimethoprim	Trimethoprim+ Sulfamethoxazol	SXT	1,25/23,75

RESULTS

Each of the twelve strains cadmium resistant- bacteria recorded multiple drug resistance Since they are resistant to at least three different families of seventeen antibiotics tested.

Ampicillin had the least impact on the strains since 91 % of the strains were resistant, followed by nalidixic (83,3%),then Celestine sulfate and Novobiocin (75%),followed by Ceftazidine and Vancomycin (66,6%),then Imipenem, Kanamycin, Ciprofloxacin,Trimethoprim+ Sulfamethoxazol and Tobrymycin with similar resistance rate of (41,6%), Gentamycin, Rifampicin and Chloramphenicol (33,3%). Aztreonam is the Antibiotic that has the most impact on bacteria with the lowest rate of resistance (25%).As shown in table 2.

Table 2: Resistance Profile of Cadmium- Resistant Bacteria to Antibiotics

Cadmium-Resistant Isolates	Resistance To
Burkholderia cepacia (YL-CS1)	GEN, AMP, IPM, SXT, V, CAZ, NA, NV.
Aeromonas hydro phyla /cavies (YL-DS1)	GEN, IPM, CS, K, RA, TE, CTX, CAZ, NA, NV
Aeromonas hydro phyla /cavies (YL-ES1)	AMP, IPM, CTX, CIP, SXT, RA, TE, CAZ, NA, NV, TOB
Aramonais hydro phyla /caviae (YL-OS1)	GEN, AMP, CTX, IPM, CIP, TE, CAZ, NA, V
Aeromonas salmonicida (YL-US1)	AMP, C, CS, SXT, NV
Pseudomonas luteola (YL-KS1)	AMP, CTX, CIP, CAZ, NA, NV, K, CS, ATM, TOB,
Pseudomonas fluorescens (YLTS1)	AMP, CTX, NA, NV, K, CS, V, TOB
Pasteurella spp (YL-SS1)	GEN, AMP, CTX, CAZ, NA, NV, K, CS, C, CIP, RA, TE, SXT, V
Shigella spp (YL-XS1)	AMP, CTX, NA, CIP, CS, C, SXT, ATM, V, TOB
Salmonella spp (YL-WS1)	AMP, CTX, TE, CAZ, C, CS, ATM, V
Bacillus cereus (YL-VS1)	AMP, CTX, IPM, NA, NV, CS, TOB,
Raoultella spp (YL-QS1)	AMP, NA, NV, CAZ, TE, K, CS, RA, V

DISCUSSIONS

Except *Bacillus cereus* which causes two types of food poisoning, the emetic and diarrheal syndromes, and a variety of local and systemic infections (Shoeing & Wong, 2005), all bacterial strains cadmium- resistant isolated from cement plant soil are non-fermentative Gram negative rods and opportunistic pathogens responsible for nosocomial infections these bacteria are also frequently isolated from hospital environment, where the main source of contamination is the water distribution network and food like fresh fruits and raw vegetables (AFAR, 2003)

Aeromonas are infectious agents for poikilothermal animals and are classified as opportunistic pathogens for humans. Gastroenteritis, wound and cellulites infections associated with exposure to water or soil, sepsis often associated with malignant pathologies, in particular hematological or hepatobiliary diseases, as well as extra-intestinal infections such as Meningitis, peritonitis, obits, endocarditic, osteomyelitis.

These bacteria penetrate the body through the digestive tract (water, food) or by break-in from wounds.

Aeromonas synthesize a wide variety of extracellular compounds that can be assimilated to virulence factors, such as fimbriae, flagella, membrane proteins, end toxins, pili, siderophores and enter toxins.

Surgical wound infections were identified after use of leeches during the postoperative period, particularly in plastic surgery or microsurgery. Indeed, *Aeromonas* are part of the digestive flora of leeches.

In the hospital, prevention is based on the monitoring of the water quality of the network, especially in summer when climatic conditions favor the multiplication of *Aeromonas*, as well as that of water in leech aquariums (Cavalier & Marty, 2008).

B. cepacia is a known cause of infections in hospitalized patients. This bacterium poses little medical risk to healthy people. However, people who have certain health problems like weakened immune systems or chronic lung diseases, particularly Cystic Fibrosis may be more susceptible to infections with *B. cepacia*.

Among the genus *Pseudomonas*, *P.aeruginosa* and *P.pseudomallei* were until recently, considered the human pathogens. However since the early 1960s, other *Pseudomonas* species have been found in clinical specimens and their number and frequency seem to be growing. *P. fluorescens* had grown in transfusion blood and caused severe to fatale reactions in the recipients (Von Graevenitz and Weinstein, 1971).

The enterobacteria are represented in our study by *Shigella*, *Raoultella* and *Salmonella*. Infections caused by enterobacteria are associated with high morbidity and mortality and cause major treatment problems due to a limited number of therapeutic alternatives. Only a few limited and often retrospective clinical studies are available, mainly on *Klebsiella pneumoniae* KPC infections, while more in vitro and animal data are available (Cattoir, 2014). Transfer of R-factors to pathogenic members of enterobacteriaceae especially *E.coli* and *Salmonella* is of grave concern particularly in controlling typhoid, diarrhea and other gastro-intestinal infections in humans (Silva *et al*., 2006).

The results obtained from the present study show that all the cadmium-resistant bacteria studied were multi-drugs resistant. In general, the antibiotic-resistance in bacteria is either natural or acquired and could be linked with that of heavy metals (Sougakoff and Tryst ram, 2003). Thus, bacterial resistance to metal ions is often coded by genes carried on plasmids encoding at the same time that of some drugs (Edward et al., 2009).

In this context, many studies demonstrated the importance of efflux pumps in antibiotic resistance associated with that of heavy metals in Gram-negative such as MexGHI-OpmD in *Pseudomonas aeruginosa* and AdeABC (RND family) efflux pump in *Acinetobacter baumannii* (Aendekerk, *et al*., 2002; Wieczorek *et al*., 2008).Based on the results obtained, strains of the genus *Aeromonas* were highly insensitive to the antibiotics tested in particular amino glycosides and β-lactamines, which is in agreement with those mentioned by certain works (Matyar *et al*., 2009).

Finally, the multi-drugs and multi-heavy metals resistance could be considered as a tool allowing these microorganisms to survive for a long time in the environment and to face the multiple stresses and made of them bacteria strongly requested to be used in the biological processes of environment detoxification**.**

The species of the genus Pseudomonas produce a layer of exopolysaccharides surrounding their cells, the protector of phagocytosis by macrophages in mammals.

This layer of exo-polysaccharide (E.P.S) allows them to form biofilms, by which they can remain stuck to the surfaces, so that it is difficult to dislodge them (Visca *et al*., 2007).E.P.S production is considered like a resistance mechanism to cadmium as reported by Halder and Basu in 2016. In fact, E.P.S are for the most part anionic compounds which can fix cations such as Mg + 2 and Ca + 2, but also heavy metals such as lead, Cadmium, Cobalt, Europium, Cesium and Strontium, which are highly toxic pollutants (Garrido *et al*., 2002)

CONCLUSIONS

The important resistance of bacteria to cadmium is beneficial to their adaptation and survey in polluted environment, but the proliferation of antibiotic-resistant bacteria, indirectly through metallic pollution selection, could poses a potential public health risk due to therapeutic failures. Measures are to be taken at the level of environmental clean up to reduce the impact and limit the propagation of resistances.

The resistance profiles observed among the different strains may be attributed to bacterial exposure to heavy metals. This characteristic makes polluted cement plant soil, a particularly suitable niche for spread of antibiotic resistance. Although antibiotics are indispensable in the treatment of infectious and other diseases, the widespread development of antibiotic resistance in the community makes it imperative that the search for new novel bioactive compounds be maintained.

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