Clinical Microbiology & Case Reports

ISSN: 2369-2111 Research Article

Screening of the Multi-resistant Bacteria Isolated from Food, Clinical Infection and Environment Water: Characterization of β- lactamases

This article was published in the following Scient Open Access Journal:
Clinical Microbiology & Case Reports
Received December 31, 2014; **Accepted** July 03, 2015; **Published** July 13, 2015

Khaldi A*, Tir Touil Meddah A and Meddah B

Laboratory of Bioconversion Microbiological Engineering and Sanitary Safety 4(LBMESS), University of Mascara-29000, Algeria

Abstract

Exerting pressure on microorganisms, misuse of antibiotics is the main epidemiological factors responsible for the emergence of multidrug-resistant bacteria (BMR) by several mechanisms, including enzyme inhibition among which the β-lactamases.

Along with the widespread use of β-lactams, β-lactamase bacteria have evolved towards diversification, broadening their spectrum of activity, and shared among many species of bacteria.

In the present study, 80 samples were analyzed from three sites, Food samples, clinical infection and environment Water. In total 115 strains were isolated from different sampling with large dominance of Gram-negative represented by 70% against 30% of Gram-positive bacteria.

In the Gram positive bacteria, *Staphylococcus aureus* was the most frequently isolated with 23%. Among the Gram negative bacteria, *E. coli* and *Klebsiella sp, Salmonella sp* were the organisms most frequently found with a prevalence of 20%, 15% and 12% respectively. However *Shigella sp* and *Providencia sp* were found at 10% and 08% in different samples. In other Gram negative bacteria *Pseudomonas aeruginosa* was isolated at 12%.

Our results indicate that all strains isolated and identified in various sites were multi-resistant to antibiotics. From different specimens (Food, clinical infection and environment water), 50% of *Staphylococcus aureus* strains isolated are presumed MRSA. In the Gram negative bacteria and from *Enterobacteriaceae* 79.46% isolated strains producing ESBL.

Keywords: β-lactamase, Environment, Food, Clinical infection, Multi-resistant bacteria

Introduction

Antimicrobial resistance is one of the major human health problems and animal medicine; it is also recognized by the WHO as an emerging public health problem. Since the phenomenon is all the more important that it concerns pathogens that can be transmitted to humans [1]. Bacterial world proved capable of adapting to antibiotics has been observed that microorganisms isolated from human and animal infections and gradually more and more frequently resisted antibiotics successively appeared [2,3].

Therapeutic control of multidrug resistant bacteria has been a major concern in the area of global public health. The riddle of the significance of antibiotics' role in nature remains unfounded due to the responses of bacteria through the manifestation of various forms of resistance following the introduction of a new antibiotic for clinical use. Gram-negative bacteria often gain their resistance through the acquisition of a resistance gene from a shared gene pool with the aid of plasmids [4,5].

This study aimed to determine the bacterial diversity from different origins food (milk, meat, Fish) Clinical infection, and water (River, sewage, sprinter) and to investigate the bacterial multi-resistance by characterization of beta lactamases.

Materials and Methods

Samples collection

This study was conducted between March and September 2013 from different region in western north of Algeria. A total of 85 samples were collected from Food (n=34), clinical infection (n=15) and environment water (n=36) (Table 1). There were transported to the laboratory and stored at – 20°C until further analysis.

***Corresponding authors:** Khaldi Amina, PhD student Laboratory of Bioconversion Microbiological Engineering and Sanitary Safety (LBMESS), University of Mascara-29000, Algeria, Tel: +213552982143, Fax: +21345707003, Email: amkhaldia@hotmail.com

Nature of sample	origin	Collection area	Collection time
Food	White meat (n=6)	Mascara	16/04/2013
	Intestines of sheep (n=5)	Saida	21/04/2013
	Intestines of cow (n=5)	Sidi Bel Abbès	28/04/2013
	Poultry intestines (chickens) (n=6)	Mascara	14/05/2013
	raw milk (n=6)	Saida	21/04/2013
	Fish (n=6)	Sidi Bel Abbès	04/05/2013
Clinical infection	urinary infection (n=5)	Sidi Bel Abbès	23/03/2013
	Bone pus (n=5)	Sidi Bel Abbès	23/03/2013
	Pocket (n=5)	Sidi Bel Abbès	23/03/2013
Environment Water	Wastewater (n= 8)	Mascara	26/03/2013
	Wastewater (n= 8)	Saida	21/04/2013
	Wastewater (n=8)	Sidi Bel Abbès	04/06/2013
	River (n=6)	Sidi Bel Abbès	12/09/2013
	Springer Water (n=6)	Mascara	14/09/2013

Table 1: Samples, Origin and Area and Time collection of investigated studies.

Bacterial isolation and identification

All samples from food, Clinical infection, and Environment water were prepared by diluting scraped cell mass in 0.85% NaCl sterile solution and then analyzed for aerobic bacterial content by cultures on a series of non-selective and selective media (Blood agar, Chapman medium, Hektoen medium, Nutritive agar medium. Plates were incubated at 37°C for 24 and 48 h, grown colonies and pure cultures were identified by recommendation of Bergy's [6] using standard morphological, Gram coloration and biochemical methods with commercials kits (API Staph, API 20 E Biomerieux, Marcy l'Etoile, France). The tests were conducted twice for each sample and the mean of Colony Forming Unit (CFU) count was determined. The microbial strains were maintained on agar slant at +4°C until analysis.

Inoculums' preparation

Nutriment broth [7] was used for growing strains and diluting suspensions. Bacterial strains were grown to exponential phase in nutriment broth at 37°C for 18 h and adjusted to a final density of $2x10^8$ CFU by diluting fresh cultures and comparison to Mac Farland standards (OD_{650}=0.7) [8].

Antibiotic susceptibility testing

Resistance towards antibiotics was assessed for each strain with the disc diffusion method [9] and bacterial growth on Muller Hilton Agar plates. The antibiotic tested for Staphylococaceae, were Oxacillin (10 µg), Erythromycin (15 µg), Spriramycin (10 µg), Chloramphenicol (30 µg), Tetracyclin (30 µg), for Enterobacteriaceae were Ampicillin (10 µg), Gentamicin (10 µg), Aztreonam (30 µg), Colistin (10 µg), Tetracyclin (30 µg), Chloramphenicol (30 µg), for Clostridiaceae were Cefazolin (10 µg) Nalidixic Acid (30 µg), Amoxicillin (10 µg), Colistin (10 µg).

Staphylococcus aureus resistant Methicillin (MRSA) search

Dip a sterile swab into the bacterial suspension standardized at 10^6 CFU / ml. The swab is rubbed on the agar surface of the screen (Muller Hinton). Submit a oxacillin 10 µg the center of the box hard and incubated for 24 h at 37°C isolated in the presence of the inhibition zone around the disk colonies resistant MRSA and heterogeneous [10] (Photo 01).

β- lactamases search in gram negative bacteria

Phenotypic demonstration of the presence of a β-lactamase extended spectrum in Enterobacteriaceae is to highlight an image of a disk synergy between third-generation cephalosporin and clavulanic acid. Apply on Mueller Hinton agar [10] previously seeded with the test strain, a disc of cephalosporin 3rd generation: (aztreonam (ATM) or ceftazidime (CAZ), or cefixime (CFM) or cefotaxime (CTX), after incubation for 18 h at 37°C (Photo 02).

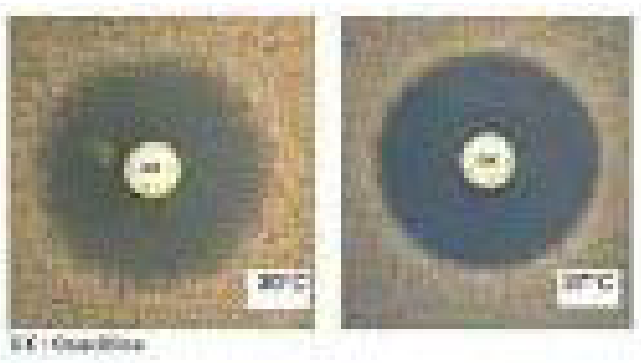

Photo 1: *Staphylococcus aureus* resistant for Methicillin

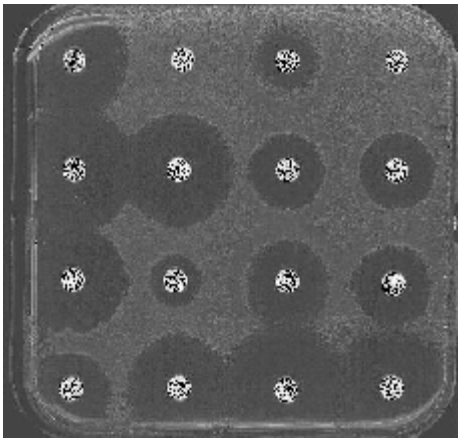

Photo 2: β-lactamase extended spectrum in Gram Negative bacteria

Statistical Analysis

All experiments were made in duplicate. The tests were conducted twice for each sample and the mean of Colony Forming Unit (CFU) count was determined. The results are presented as the mean ± SD. The frequency of strains isolated and antibiotic sensibility test were calculated as percentage.

Results

Pathogens bacteria isolated from different samples

In Total 115 strains were isolated from different samples (Food, clinical infection, environment water). According to the results, a large dominance of Gram-negative represented by 70% were founded against 30% of Gram-positive bacteria (Figure 1).

We notice that in the Gram positive bacteria, *Staphylococcus aureus* was the most frequently isolated with 23% (Figure 2). Among the Gram negative bacteria, *E. coli* and *Klebsiella sp, Salmonella sp* were the organisms most frequently found with a prevalence of 20%, 15% and 12% respectively (Figure 2). However *Shigella sp* and *Providencia sp* were found at 10% and 08% in different samples (Figure 2). In other Gram negative bacteria, *Pseudomonas aeruginosa* was isolated at 12% (Figure 2). This results was the same as reported in a study of Moukrad et al. [11].

Antibiotic sensitivity profile of isolated pathogens strains

From food: The profile of antibiotic in bacteria isolated from different Food shows that most Gram-negative bacilli have a total resistance to colistin and ampicillin (100%) and 95.23% to 89.47% in Tetracycline and Chloramphenicol (Figure 3a). However as all Gram-positive cocci have a high resistance to Tetracycline, Spiramycin, penicillin and oxacillin and variable resistance to other antibiotics tested. Otherwise; there has been a very high sensitivity of all germs against Gentamycin (Figure 3b).

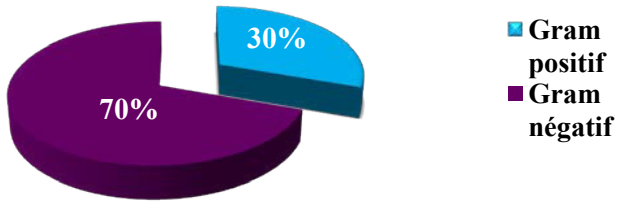

Figure 1: Frequency of pathogens species of Gram-positive and negative isolated from different Samples.

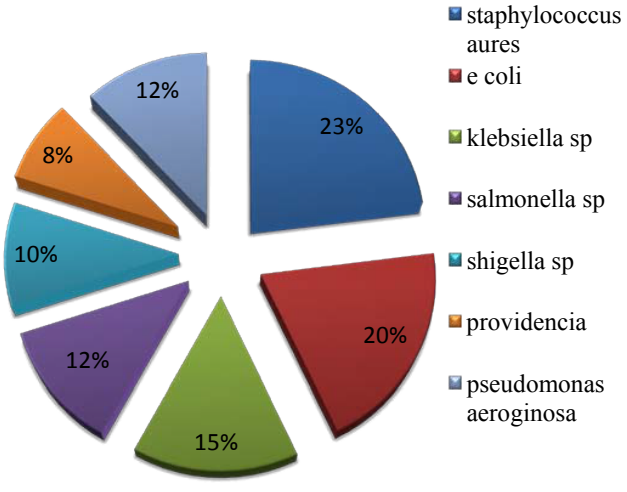

Figure 2: Frequency of pathogens bacterial strains isolated from different samples.

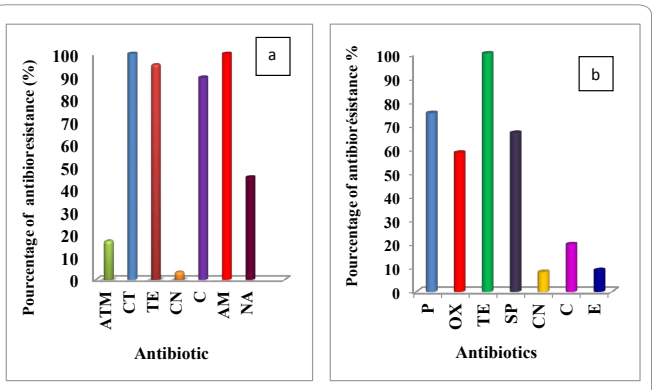

Figure 3: Percentage of antimicrobial resistance isolated from food products
a) Gram-negative bacilli b) Gram-positive cocci

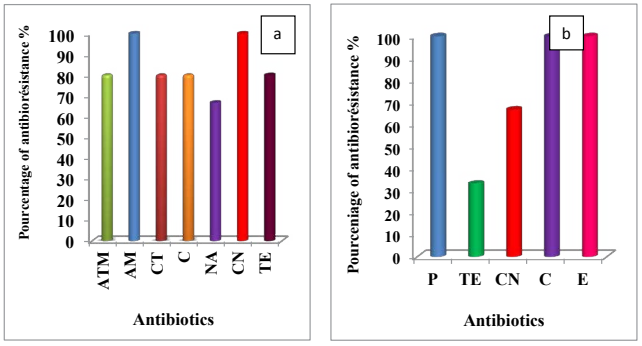

Figure 4: Percentage of antimicrobial resistance isolated from clinical infection
a) Gram-negative bacilli b) Gram-positive cocci

From clinical infection: The profile of antibiotic resistance in bacteria isolated from various clinical infection settings shows that most Gram-negative strains have a total resistance to Ampicillin and Gentamicin and 80% to the aztreonam, while they showed varying resistance to other antibiotics tested (Figure 4a).

However, all gram-positive strains have a total resistance to penicillin and chloramphenicol and erythromycin variable resistors other antibiotics tested (Figure 4b). Our results related to the susceptibility of isolated bacteria to antibiotics, show *that E. coli* isolated from the clinical infection showing a trend of increasing resistance to the majority of antibiotics, which is consistent with the results of studies in other countries such as France, the United States and Tunisia [8,9,12,13]. Klebsiella is naturally resistant to ampicillin, it has acquired other types of resistors which may act in a way giving simultaneous multi strains very high strength.

Comparing the rate of antibiotic resistance in *Klebsiella sp* to antibiotics tested is common in 1993 and 2010, shows that this bacteria has developed more resistance during this period

of time especially for ceftazidime, whose rate resistance has increased from 00-39%, and for the fluoroquinolone from 10 to 27%) which showed a highly correlation with our study [11].

The evolution of bacterial resistance to antibiotics over time is illustrated by comparing the profiles of resistances implicated the nosocomial infections. In this study we have shown that in a time interval, the increase of the resistance against a various families of antibiotics is multiplied which were confirmed by Rhazi Filali [13]. The acquisition by bacteria, means to fight against the lethal effect of antibiotics or genotypic changes that result, progress in the same direction as this resistance making the bacteria more virulent [11].

From environment water: The profile of antibiotic resistance among the different pathogens isolated from the environment water shows that most Gram-negative strains have a high resistance to colistin (84%), Tetracycline (84.61%), ampicillin (69.59%) and to Chloramphenicol (65.51%) respectively, then they showed varying resistance to other antibiotics tested (Figure 5a). However, all Gram-positive strains have a total resistance to erythromycin (100%) and important for Spiramycin (81.81%) and to penicillin (75%) while variable resistors are presented to other antibiotics tested (Figure 5b).

Detection of MRSA

Among strains of *Staphylococcus aureus* isolated from different specimen (food, clinical infection and environment water) 50% are presumed MRSA (Photo 03). MRSA is resistant to methicillin by acquiring a gene producing a modified penicillin binding protein (PBP2a). This protein is encoded by the mecA gene locates on a mobile genetic element. It acts as transpeptidase linking peptidogly can essential to the membrane structure of the bacterial cell [14]. The PBP2a are different from regular PLP their very low affinity for antibiotics with a β-lactam ring. For this reason, penicillins, cephalosporins and other β-lactam antibiotics are not effective against MRSA, and cross-resistance occurs with clindamycin, the Carbapenems, macrolides and tetracyclines. Vancomycin is a possible alternative first-line [14]. Since the implementation of a Federal Program Nosocomial Infection Surveillance [15] in 1995, the incidence of MRSA in hospitals has increased by 20, from 0.46 per 1,000 admissions in 1995 to 9.5 per 1000 admissions in 2009 [15].

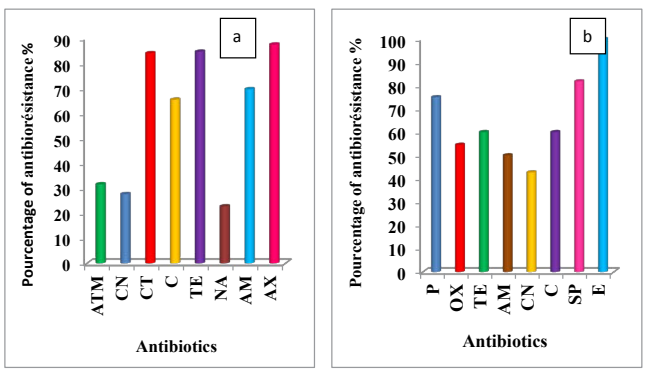

Figure 5: Percentage of antimicrobial resistance isolated from environment water
a) Gram-negative bacilli b) Gram-positive cocci

Photo 3: Detection of staphylococcus aureus resistant for methicillin.

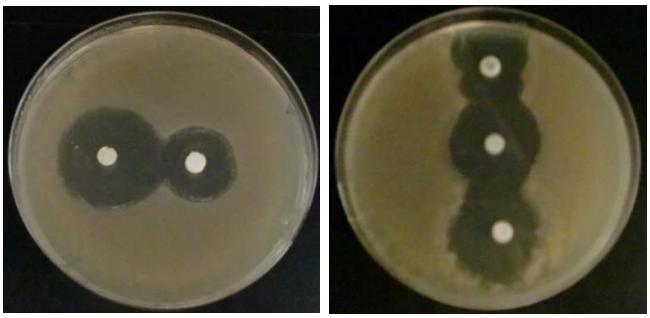

Photo 4: Production of ESBL among Gram-negative bacilli.

Detection of beta-lactamase in Gram negative bacteria

The result of a positive test is the inhibition zone around the discs cefixime or aztreonam in the presence of amoxicillin and clavulanic acid [16]. All strains isolated from different specimen (Food, clinical infection, environment water) and resistances to antibiotics were tested for the production of beta-lactamase using the spread spectrum method previously described double halo. The results indicates that the most isolated strains (79.46%) producing ESBL (Photo 4), which are mainly *Escherichia coli*, *Klebsiella pneumoniae*, *Hafnia alvei, Enterobacter aerogenes*, *Pseudomonas aeruginosa*. During antibiotic therapy, the acquisition of resistance determinant and the selection of resistant subpopulations in patients initially infected with a susceptible strain, presents a major problem [17].

The present study has shown that all strains are multi-resistants. Previous studies have shown that ESBL-mediating plasmids may carry more than one beta-lactamase gene and that they may be responsible for high-level beta-lactamase resistance phenotypes [18]. The major risk in the beta-lactamase genes particularly in food samples and in environment water organisms could have opportunities for environmental dissemination and possibly human exposure and transmission.

The direct and indirect costs associated with antibiotic resistance; promote national coordination and development of action plans to respect the measures of hygiene, combat antibiotic resistance; promote the prudent use of antibiotics and the systematic implementation of infection control measures for the prevention and treatment for reduce morbidity, mortality.

The use of antibiotics in human health and animal, including the impact on the food chain; review the teaching of prudent use of antibiotics in the faculties of medical sciences, veterinary and life, and implement effective policies.

Conclusion

This study clearly demonstrates the remarkable presence of pathogens bacteria in different samples which can be implicated into quantitative risk assessment. Our study over a short period raised significant data on the alarming development of resistance and the emergence of multidrug-resistant bacteria to antibiotics. Indeed all of the isolated bacterial strains have a significant increasing trend with multidrug resistance over time as a consequence of improper use of antibiotics.

The testing for beta-lactamase reveal the presence of ESBL of penicillinase and carbapenemase in most organisms isolated. However, resistance by producing penicillinase is the type o resistance the most common.

In general, to reduce the selection pressure of antibiotics that encourages the emergence, proliferation and spread of SARM and ESBL in *Enterobacteriaceae*, it should reduce the volume of antibiotics used in humans by intensifying actions in part of the antibiotic level. We must, in particular, introduce, next to the concept of "good use", the concept of "any use". A strong proposal to advance in this area is to define, document and make the situations in which it is recommended not to prescribe antibiotics.

Finally, it will be interesting to eliciting a possible relation between pathogens bacteria isolated from different site which has considerable implication for consumers' hearth.

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