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ISOLATION AND IDENTIFICATION OF PSEUDOMONAS AERUGINOSA STRAINS PRODUCING β-LACTAMASES (ESBL) AND CARBAPENEMASES (MBL) OF HUMAN ORIGIN

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Abstract
The community of medical veterinarians and humans draw attention to the microorganisms with potential pathogens that are both common and multiresistant to the latest antibiotics. Bacteria common to both animals and people favor the cross transmission of these strains with a major public health risk. Pseudomonas aeruginosa, a motile, non-fermenting Gram-negative bacterium, is an important opportunistic animal and human pathogen that causes acute and chronic infections in immunocompromised patients.
Pseudomonas aeruginosa has acquired several mechanisms of resistance to multiple groups of antibiotic agents.
The isolation and identification of Ps. aeruginosa producing ESBL and MBL may often be challenging to microbiology laboratories, the level of expression of β-lactamases and MBL can affect the performance of phenotypic tests, and the lack of synergy might be due to a very high level of expression overcoming the effect of the inhibitors resulting in false-negative results. Ps. aeruginosa from different purulent collections has an atypical appearance, even a specific pigment that is not present for all the strains.
These investigations are part of a larger research study, aimed at highlighting the strains of Pseudomonas aeruginosa common resistance genes in humans and animals. In this study, 93 Ps. aeruginosa strains were collected from pediatric patients. In order to establish resistance, profiling samples were isolated and identified for the production of β-lactamase medium Brilliance ESBL AGAR (Oxoid) and for the production of MBL was tested Imipenem with EDTA.

Key word: *Ps. aeruginosa, ESBL, MBL, isolation, identification.*

INTRODUCTION

Pseudomonas aeruginosa is a common opportunistic and nosocomial pathogen that causes severe infections with a hight mortality rate, especially in immunocompromised patients or those with underlying disease (Poole, 2011), and is a leading nosocomial Gram-negative pathogen well known for its intrinsic as well as extraordinary ability to develop resistance to various antimicrobial agents, remain a significant challenge to clinicians, given that therapeutic options are limited to a handful of agents in three major classes.

It is a gram-negative, aerobic, citrate, catalase, and oxidase positive. It is found in soil, water, skin flora, and most man-made environments throughout the world (Cătălin Carp-Cărare, 2014). *Ps. aeruginosa* secretes a variety of pigments, including pyocyanin, pyoverdine and pyorubin (Palmer KL, 2007).

ESBL (Extended spectrum β-lactamases)

ESBLs have an extended substrate profile that cause hydrolysis of cephalosporins, penicillins and aztreonam and are inhibited by β-lactamase inhibitors, such as clavulanate, tazobactam and sulbactam. ESBLs are commonly produced by Klebsiella species and Escherichia coli; but also occur in other Gram negative bacteria, including *Enterobacter, Salmonella, Proteus, Serratia marcescens, Pseudomonas aeruginosa, Burkholderia, Acinetobacter* species, etc.

Carbapenemases

These include β-lactamases which cause carbapenem hydrolysis, with elevated carbapenem MICs and they belonged to molecular classes A, B and D. Molecular classes A, C and D include the β-lactamases with serine at their active site, whereas class

B β-lactamases are all metalloenzymes with an active site zinc (Queenan & Bush, 2007).
The present study was undertaken with the aim to study *Pseudomonas aeruginosa* with special reference to β-lactamase production isolated in the pediatric hospital. To follow the aim and objectives were taken is to study the antibiotic susceptibility profile of extended Spectrum β-lactamases (ESBL), and carbapenemases (MBL) producing *Ps. aeruginosa* strains isolated.

MATERIALS AND METHODS

A total number of 93 *Ps. aeruginosa* strains were isolated from different clinical samples e.g. urine, pus and wound swab, blood, catheter tips, endotracheal tube secretions, etc. Samples from patients that high clinical suspicion of infection was performed by medical staff sectors involved in this study, respecting general rules strictly harvesting products for bacteriological examination.
The performances of isolation media in stimulating vary production pigment. The best results were obtained on nutrient agar base medium for Pseudomonas (Merck, Oxoid) with cetrimide (CN). Biochemical confirmation of the strains in this study was done by using the API 20 NE and RapID NF Plus tests, using both tests to highlight the advantages of Rapid NF Plus tests. (Ciocan O.A., 2014).
Ps. aeruginosa ATCC 27853 was used for quality control of susceptibility testing.

Phenotypic identification of ESBL producing isolates

Brilliance ESBL are chromogenic media designed for selective isolation and presumptive identification of ESBL-producing GNB (Gram-negative bacteria), based on a rich nutrient capacity with a selective mixture of antibiotics, including cefpodoxime. This antibiotic is recognised as being the marker of choice for this resistance mechanism (Paterson DL, 2005).
Literature data concerning rapid screening for carriage of ESBL- producing GNB among high-risk patients by use of commercially available, selective (chromogenic) media supplemented with one or more antimicrobial agent(s) are limited (Glupczynski et al., 2007, Huang et al., 2010; Paniagua et al., 2010; Reglier-Poupet et al., 2008; Saito et al., 2010).
After vortexing (5″), 100 µL of homogenized ESwab's (Copan Diagnostics, Murrieta, CA, USA) Liquid Amies suspension medium was inoculated Brilliance ESBL (Oxoid, Hampshire, United Kingdom). Samples were incubated at 35 °C ± 2 °C in ambient air and examined after 18 to 24 h and 42 to 48 h of incubation.
Phenotypic identification of ESBL producing isolates with special media have been carried out using DDST screening method. Anti-biogram disks containing ceftazidime (30 lg), cefotaxime (30 lg), ceftazidime (30 lg) + clavulanic acid (10 lg) and cefotaxime (30 lg) + clavulanic (10 lg) were used. Pairs of disks (ceftazidime with ceftazidime/clavulanic acid and cefotaxime with cefotaxime /clavulanic) were placed on Muller-Hinton agar medium (Merck, Germany) with 15 mm space between them. According to the CLSI criteria and manufacturer instruction, the P5 mm inhi-bition zone of growth in ceftazidime/ clavulanic acid and cefotaxime/clavulanic than ceftazidime and cefotaxime was regarded as an isolate that is producing ESBLs.

Phenotypic MBL detection

For determination of phenotypic MBL production among the bacterial isolates, we test disks containing imipenem plus EDTA (Oxoid, UK). The test procedure was performed according to the manufacturer's manual, growth inhibition zones in the presence of EDTA is regarded as a positive result, were tested just the strains that was resistant to imipenem.

RESULTS AND DISCUSSIONS

Phenotypic identification of ESBL producing isolates have been carried out using DDST screening method. From total of 93 samples, 92,47% (86 strains) *Pseudomonas aeruginosa* isolates identified to be produce ESBL enzymes.
The resistance rate to ceftazidime was 100 %.
For determination of phenotypic MBL production among isolates, disks containing

imipenem with EDTA were used. 35,48% (33 strains) out of 93 *Pseudomonas aeruginosa* isolates were MBL producing.

Production of carbapenem-hydrolyzing b-lactamases, also called carbapenemases, is one of the significant mechanisms of carbapenem resistance, in which Methalo β-lactamases (MBLs) possess the principal role in drug resistance against carbapenems (Poirel and Nordmann, 2006). Also, the ESBLs, play an important role in resistance against later generation cephalosporins (Zhanel et al., 2013).

In this study, the prevalence of ESBLs and MBLs encoding phenotyp tests and drug resistance against *Pseudomonas aeruginosa* isolates has been investigated showing a high resistance rate among the antibiotics.

CONCLUSIONS

For a good and accurate evidentiation of Pseudomonas aeruginosa strains we recommend isolating the strains on Pseudomonas agar base using cetrimide (CN - OXOID) as selective supplement: the pigment appears highly pronounced compared to other growth media, as for biochemical confirmation we recommend the RapID NF Plus test since it provides the main advantage of reducing the identification time to 4 h.

The results showed that most of the Pseudomonas aeruginosa isolates were producing MBLs (34,48%) and ESBLs (92,47%).

I used two phenotypic methods for detection of ESBL-producing strains to verify the specificity of the Brilliance ESBL, chromogenic media designed for selective isolation and presumptive identification of ESBL, but also to shorten working time, and the necessary materials, in conclusion we can say that using a particular environment is more rapid detection method for ESBLs strains.

The DDST and the combined disk test (CDT) are the most commonly used formats of ESBL and MBL detection assays. The DDST uses a b-lactam disk placed closed to a disk with a given amount of an MBL inhibitor. The formation of a synergy pattern is indicative of ESBL/MBL production. Alternatively, in the CDT variant, the b-lactam disk is potentiated with an inhibitor, and the diameter of its inhibition zone is then compared with that of the b-lactam disk alone. An increase in the inhibition zone diameter above a predefined cut-off value indicates ESBL/MBL activity.

ESBL/MBL-producing bacteria have now spread all over the world. Infections caused by those bacteria are difficult to treat. Therefore, there is an urgent need for accurate and fast detection of ESBL and MBL in diagnostic laboratories.

Molecular techniques remain the reference standard for the precise identification of ESBL and MBL strains, PCR is the fastest way to determine which tipe of ESBL/MBL is present.

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