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STUDY OF CARBAPENEMS PHENOTYPIC RESISTANCE IN PSEUDOMONAS AERUGINOSA ISOLATED FROM BURNS IN MISAN PROVINCE

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ABSTRACT

The study aimed to evaluate the distribution of *Pseudomonas aeruginosa* isolated from Burns and study of the Phenotypic resistance to carbapenems which widely used in units of burns. Total number of 226 samples were collected from burns during period from October, 2013 till November, 2014. All samples were routinely collected, cultured on MacConky ,Chocolate and blood agar, the identified bacteria were distributed as follow: *Pseudomonas aeruginosa* 40(15%), Klebsiella spp. 38(14.28%), Staphylococcus spp. 39(14.66%), Protus spp. 30(11.27%), E.coli 100(37.59%), Enterococcus spp. 3(1.1%), Streptococcus spp. 5(1.88%), Enterobacter spp. 11(4.13%). To study of phenotypic resistance patterns of *Pseudomonas aeruginosa*, twenty- two of identified bacteria were entrant to Vitak 2 system where the Minimum Inhibitory Concentration(MIC) was used .The results showed that resistance to Imipenem occurred in 9.09% of isolates while 4.5% were resistant to meropenem. Our conclusion was that there was resistance to carbapenems due to pressure obtained by using these antimicrobial agents which complicated the choice of drugs for treatment in units of burns.

INTRODUCTION

Pseudomonas aeruginosa is an aerobic Gram-negative rod . It is widely distributed in nature and can acclimatize to many environments, it can be isolated from any probable source within hospitals (Brooks *et al*., 2007). It is an important cause of community acquired and hospital-acquired infections. Thisbacteria have been associated with high morbidity and mortality when compared with other bacterial pathogens (Brusselaers *et al*., 2011). *Pseudomonas. aeruginosa* infections are problematic to treat because of high intrinsic resistant to many antibiotics (Multi-drug resistant) and a high threat of occurrence of resistance during therapy (Livermore, 2012).

Carbapenems are a group of β-lactam antibiotics with a broad spectrum of antibacterial activity. Their structure makes them highly resistant for most β-lactamases (Krisztina *et al*., 2011). They include meropenem and impenem, which are among the few therapeutic choices still available for treating infections caused by *P. aeruginosa* (Lim *et al*.,2011). Carbapenems

resistance in *P. aeruginosa* was stated to increase steadily over the years thru the world, but the contribution of different carbapenems resistance mechanisms is not well established (Khuntayaporn *et al*., 2012).

The study amied to:

1-Isolation and Identfication of *P. aeruginosa* from burn patients

2- Detection of of *P. aeruginosa* resistance to carbapenems using phenotypic test.

Material and methods

Samples Collection

A total of 266 samples were collected during the period from 1/10 /2013 to 1/4/2014 from patients visited/or admitted to AL-sader hospital in Misan province. Samples which were collected by sterile swabs and containers had been cultured on macConkey agar, chocolate agar and blood agar, to get pure colonies subculture done on macConkey agar, incunated for overnight at 37C^{o}.

Isolation and Identification

The identified isolates which had been cultured on MacConky agar, chocolate agar and Blood agar,. MacConkey's agar medium is specially made to distinguish lactose non-fermenting (pale or colorless colonies) from lactose fermenting bacteria (pink to red colonies). The identification to species level confirmed by using automated Vitek 2 system. The identification could be briefly described as following:

Suspension was prepared according to the manufacturer's recommendations of bioMérieux company by transferring sufficient number of colonies from overnight pure culture by swab and suspending the microorganism in 3.0 ml of sterile saline in a can tube . Turbidity had been adjusted to be from 0.5 to 0.63 using a turbidity meter called DensiChek. The same suspension was used in identification and antibiogram testing with VITEK-2 compact system. The identified isolates were confirmed with the automated VITEK-2 compact system by using GN-ID cards. This system identifies an organism via a methodology based on the characteristics of the data and knowledge about the organism and reaction being analyzed.

GN-ID cards were inoculated with microorganism suspensions using an integrated vacuum apparatus. A test tube containing the microorganism suspension was placed into a special

rack (cassette) and the identification card is placed in the neigh boring slot while inserting the transfer tube into the corresponding suspension tube.

The cassette can accommodate up to 10 tests with VITEK-2 compact system. The filled cassette was placed manually into a vacuum chamber station. After the vacuum was applied and air was re-introduced into the station, the organism suspension was forced through the transfer tube into micro-channels that fill all the test wells.

Minimum Inhibitory Concentration (MIC) by Vitek system. Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed with the automated VITEK-2 compact system based on MIC technique determination by using AST-N82 cards. This card contained the following antibiotics, Ampicillin, Amoxicillin/ clavulanic acid, Ampicillin/ sulbactam , piperacillin/tazobactam, cefazolin, cefepime, ceftazidime, ceftriaxone, aztreonam, imipenem, meropenem, amikacin, tobramycin, gentamicin, ciprofloxacin, trimethoprim-sulfamethoxazole, tigecycline, nitrofurantoin.

AST-N82 cards were inoculated in the same manner as described .VITEK-2 compact system evaluates each organism's growth pattern in the presence of the antimicrobial in relation to growth control well. Several parameters based on the growth characteristics observed are used to provide appropriate input for MIC calculations. The MIC result must be linked to an organism identification to determine a category interpretation. A category interpretation of MIC will be reported according to the interpretations defined by Global 2012.

Results

Isolates identification

The results of identification by Vitek 2 system revealed that out of 266 of bacteria recovered from burns, *Psedomonas aeurginosa* represent 40(15%) of identified isolates as shown in Table (1).

Table (1) Number and percentage of Bacteria isolated of units burn

Bacteria	Number	%
Psedomonas aeurginosa	40	15
E.coli	100	37.59
Klebsieila spp.	38	14.28
Staphylococcus spp.	39	14.66
Streptococcus spp.	5	1.88
Enterococcus spp.	3	1.1
Enterobacter spp.	11	4.13
Protus spp.	30	11.27
Total	266	100

As shown in Table (2) which explains the bio number, biochemical tests of Vitek 2 system and the percentage of probability of identification obtained by Vitek 2 system.

Table(2): Bio number and probability of Pseudomonas aeruginosa of Vitek 2 system

Isolate No.	Bio number	Probability
1	0043051103500372	99%
2	0043511103500272	95%
3	0043051103500270	95%
4	0043051103500372	99%
5	0043051103500252	99%
6	0043051103500352	99%
7	0043051103500352	99%
8	0043053003500040	99%
9	0043053003500250	99%
10	0043051003500252	99%
11	0043053003500250	99%
12	000345003540352	96%
13	0003453104540352	96%
14	0043051003500352	99%
15	0043051003500252	99%
16	0043051103500272	95%
17	0043051103500352	99%
18	0043041003500252	97%
19	003453103500040	99%
20	0043051003500252	99%
21	2023110341552211	95%
22	0043053003500250	99%

Susceptibility profiles of isolates

The Table (3) explains the susceptibility profile of different antibiotics tested by Vitek 2 system by mean of Minimum Inhibitory Concentration(MIC), the Table Shows the susceptibility patterns , numbers of isolates related to each one and the percent of susceptibility.

Table (3) Susceptibility profile of Pseedomonas aeruginosa to different antibiotic classes used in Vitek 2 system (n = 22) .

Antibiotic	**Sensitive**	%	**Intermediate**	**%**	**Resistance**	**%**
Ampicillin	6	27.3	0	0	16	72.72
Amoxicillin/clavlanic acid	5	22.72	2	9.09	15	68.18
Ampicillin/Sulbactam	3	13.63	0	0	19	86.36
Piperacillin/Tazobactam	14	63.63	2	9.09	6	27.3
Cefazoiln	2	9.09	0	0	20	90.9
ceftazidime	15	68.18	2	9.09	5	22.7
Ceftricaxone	3	13.63	1	4.5	18	81.8
Cefepime	15	68.18	1	4.5	6	27.2
Meropenam	21	95.45	0	0	1	4.5
Imipenem	19	86.36	1	4.5	2	9.09
Gentamicn	15	68.18	1	4.5	6	27.3
Tobramycin	15	68.18	1	4.5	6	27.3
Ciprofloxacin	14	63.63	1	4.5	7	31.8
Nitrofurantion	11	50	0	0	11	50
Trimethoprim/sulfamethoxazole	2	9.09	0	0	20	90.9
Tigecycline	17	77.27	1	4.5	4	18.18
Aztreonam	1	4.5	1	4.5	20	90.90
Amikacin	20	90.90	0	0	2	9.09

Discussion

Microbiological Analysis and Isolation Rate

In this investigation, a total number of 266 were subjected to bacteriological examination for detection and isolation of *P. aeruginosa* isolates. Incidence of *P. aeruginosa* among the examined samples was 40 positive isolates with a percentage of 15 % (Table 1) and Table (2) which explains the bio numbers and percentage of probability of identification obtained by Vitek 2 system.

Pseudomonas aeruginosa is an extremely adaptable organism that can adopt many ecological niches (Montie, 1998). It is exist in the clinical and hospital environment and has become specially infamous as a human pathogen, being the third-most-common hospital pathogen, causing approximately 10% of the 2 million hospital infections in the United States annually (Robert *et al.*, 2002). In 2003, *P. aeruginosa* was stated to be the most commonly isolated Gram-negative bacteria (18.1%) for nosocomial pneumonia in the United States (Gaynes and Edwards, 2005). According to the source of samples, present study showed the presence of *P.aerugionsa aeruginosa* was as two of the most Gram-negative bacteria isolated from clinical samples (14.8%). A few studies designated infection with *P-aerugionsa.* in maysan however, the same condition was described by Belal (2010) and by Al-Muhannak (2010) who recovered 26.1% and 30.5% *P. aeruginosa* isolates from different clinical

samples in Najaf hospitals respectively. As confirmed in other local studies Al-Janabi (2011) stated that *P. aeruginosa* was isolated in a percentage of 28.2 %, in Al Dewaniyah city.

Vincent (2000) clarified in his study which conducted as a European survey of intensive care units that *P. aeruginosa* was the most frequent bacterial isolate accounting for 29% of the total isolates. In a study in Jakarta, Lucky *et al.* (2012) testified that *P. aeruginosa* was in the five most frequent Gram-negative bacteria found in clinical samples. It was the second most frequent in 2004, the third in 2005, 2007 to 2009, and the fourth in 2006 and 2010.

The burn wound typifies a site susceptible to opportunistic colonization. Though present techniques of burn wound care have significantly reduced the incidence of infections in patients with burn wounds (Pruitt *et al.,* 1998), severely burned patients may develop life-threatening infections. *P. aeruginosa* as an important life-threatening nosocomial pathogen plays a prominent role in serious infections in burn patients. Conditions for patients infected by this bacterium are predominantly problematic due to its ability to colonize wounds and rapidly in a compromised host, as well as the organism is intrinsically resistant to many drug classes and is able to acquire resistance to all effective antimicrobial drugs (Pirnay *et al.,* 2003; Ranjbarl *et al.,* 2011).

Susceptibility profile

Carbapenems are a class of β -lactam antibiotics with good antimicrobial activity against *P. aeruginosa* but the emergence and spread of acquired carbapenem resistance in this species have challenged the success of therapeutic and control efforts (Riera *et al*., 2011). In the present study activity of imipenem and meropenem to *P. aeruginosa* as shown in table (3) were 9.09% and 4.5% respectively versus to others which found imipenem had a better activity than meropenem (Gupta *et al.,* 2006).

This also agreed with the results reported by Tan *et al.* (2008) who found that meropenem was better than impenem. When compared to previous studies carried out in Najaf, current finding indicated that the rate of resistance against imipenem has lower in recent study compared with previously reported by Al-Muhannak (2010), who found the resistance rate was 16.9%. On the other hand, the resistance was higher as compared with Belal (2010) who found all *P. aeruginosa* clinical isolates tested were susceptible to imipenem. Carbapenems are a drug that is not readily available and its cost is also prohibitive, however, this group of antibiotics has been reported to be very active against *P. aeruginosa* in a number of recent

studies. Furthermore imipenem has been informed to be very active against *P. aeruginosa* in a number of studies (Reinert *et al*., 2007; Yasmin,2012).

This study suggested high prevalence of *Pseudomonas aeruginosa* as clinical isolates of burns, this declaring supported by its recording as second most frequent bacterium recovered from burns and concluded high rate of resistance to carbapenems which strongly push towards necessary to diminishing the selective pressure otherwise drug resistance occur which make the decision of drug choosing in patients of intensive care units very difficult.

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