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Pseudomonas aeruginosa identification and MIC assessment from clinical isolates

Hany Hashem^{a,*}, Amro Hanora^b, Salah Abdalla^c, Alaa Shawky^d, Alaa Saad^e

^a Microbiology and Immunology Department, Faculty of Pharmacy Suez Canal University Egypt.

^b Microbiology and Immunology Department, Faculty of Pharmacy, Qassim University, KSA.

^c Microbiology and Immunology Department, Faculty of Pharmacy, Cairo University, Egypt.

^d Clinical Pathology Department, Faculty of Medicine, Suez Canal University, Egypt.

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Abstract

Pseudomonas aeruginosa is a common and major opportunistic human pathogen, it causes many and dangerous infectious diseases like cystic fibrosis, wounds, burns inflammation, urinary tract infection, other many infections otitis external, nosocomial infection and causes bacteremia. A main problem in *P. aeruginosa* infection that exhibits a high degree of resistance to a broad spectrum of antibiotics. A total of 147 patients of *Pseudomonas aeruginosa* were isolated from Ismailia hospitals. The clinical isolates were collected from wound, sputum and urine. Carbapenem sensitivity was performed per Kirby-Bauer disk diffusion method and minimum inhibitory concentration (MIC) was performed towards meropenem and imipenem resistant isolates by agar dilution methods. Imipenem and meropenem sensitivity result were 91% and 70%. MICs were carried out for carbapenem resistant isolates. Furthermore *P. aeruginosa* screening should be managed and antibiotic abuse should be monitored.

Keywords

Pseudomonas aeruginosa
Identification
MIC

1. Introduction

P. aeruginosa is an aerobic non-spore forming Gram-negative rod with remarkable adaptable capacity to survive and persist under a broad range of environmental conditions (Singh et al., 2010; Blanc et al., 2007; Dworkin et al., 2006) *P. aeruginosa* is a common opportunistic human pathogen acquired in both the hospital and community setting (Driscoll et al., 2007). Commercial test systems and other phenotype-based identification methods may cause misidentify of *P. aeruginosa* (Qin et al., 2003; Shelly et al., 2000). Identification is often further upset by the presence of other closely related non-fermenting gram-negative bacilli, including other *Pseudomonas* species (Burns et al., 1998). The potential for

misidentification of this species from culture presents an obstacle to patient management, particularly with respect to antimicrobial therapy and infection control (Morales et al., 2012).

2. Materials and Methods

2.1. Bacterial isolates

Our study included one hundred and forty-seven *Pseudomonas* spp. (147), were collected from Suez Canal University Hospital in Ismailia, Egypt, with different sources of infections. *Pseudomonas* samples were isolated by standard microbiological procedures, cultured on ceftrimide agar (selective media), Identified using API 20NE (BioMérieux, France), and stored in Luria-Bertani broth medium (Merck, Germany) containing 30% glycerol at -80°C.

2.2 Minimum inhibitory concentration (MIC)

MIC to imipenem and meropenem was done by

*Corresponding author

Business Tel: +2-011-14888862

Fax: +2-064-3230741

E-mail: hany2515@yahoo.com

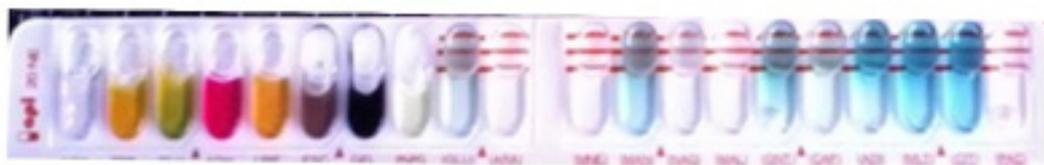


Figure 1: Identification of the isolated strains by API 20NE strip

agar dilution technique. Commercial preparation of imipenem (500 mg powder, Manufacturers: Glaxo Smithkline, Egypt Ltd) and meropenem (500 mg powder, Astra Zeneca pharma, Egypt Ltd) were taken for the study, Powders were graded serially to obtain drug concentrations ranging from (1024 – 0.125 $\mu\text{g}/\text{ml}$). One milliliter of appropriate dilution of imipenem and 19 ml of Mueller Hinton agar cooled to 55°C was added to the corresponding labeled Petri dish after mixing thoroughly. The inoculum was prepared from overnight grown cultures of the test strains. The turbidity was matched to 0.5 Mc Farland's standards. Each plate was divided into sixteen quadrants and 2 μl of inoculum was delivered to the surface of agar plate. The plates were incubated for 16 to 18 h at 37°C. The highest dilution showing no visible growth was taken as minimum inhibitory concentration (MIC). *P. aeruginosa* ATCC 90271 was used as a control.

3. Results and Discussion

One hundred and forty-seven (147) *Pseudomonas* isolates were cultured on selective *Pseudomonas* media (cetrimide agar) and identified via biochemical

tests. The analyzed results of API 20NE tests by API 20NE software showed that 125 isolated strains were confirmed to be *P. aeruginosa* out of 147 *Pseudomonas* spp. (Figure 1) isolates were *Burkholderia cepacia*. The results of MICs of carbapenem resistant *P. aeruginosa* isolates (Figure 2 & 3) were interpreted per the interpretative guidelines of the CLSI (Table 1).

In our investigation, imipenem MIC were, one isolate was found to have an MIC of 1 $\mu\text{g}/\text{ml}$, 8 isolates had an MIC value of 2 $\mu\text{g}/\text{ml}$, 4 isolates had an MIC of 4 $\mu\text{g}/\text{ml}$, 7 isolates had an MIC of 8 $\mu\text{g}/\text{ml}$, 7 isolates had an MIC of 16 $\mu\text{g}/\text{ml}$, 3 isolates had an MIC of 32 $\mu\text{g}/\text{ml}$, 6 isolates had an MIC of 64 $\mu\text{g}/\text{ml}$, and one isolate for each had MIC of 128 $\mu\text{g}/\text{ml}$, 256 $\mu\text{g}/\text{ml}$, and 512 $\mu\text{g}/\text{ml}$ against Imipenem. In 2012 Balank et al observed that MIC was <2 $\mu\text{g}/\text{ml}$, 25 isolates (12.5%) were intermediate sensitive with an MIC of 2 $\mu\text{g}/\text{ml}$ and 8 isolates (4%) were found to be resistant with 7 having MIC of 4 $\mu\text{g}/\text{ml}$ and one with an MIC of 8 $\mu\text{g}/\text{ml}$.

In our study MIC for meropenem, 2 isolates had an

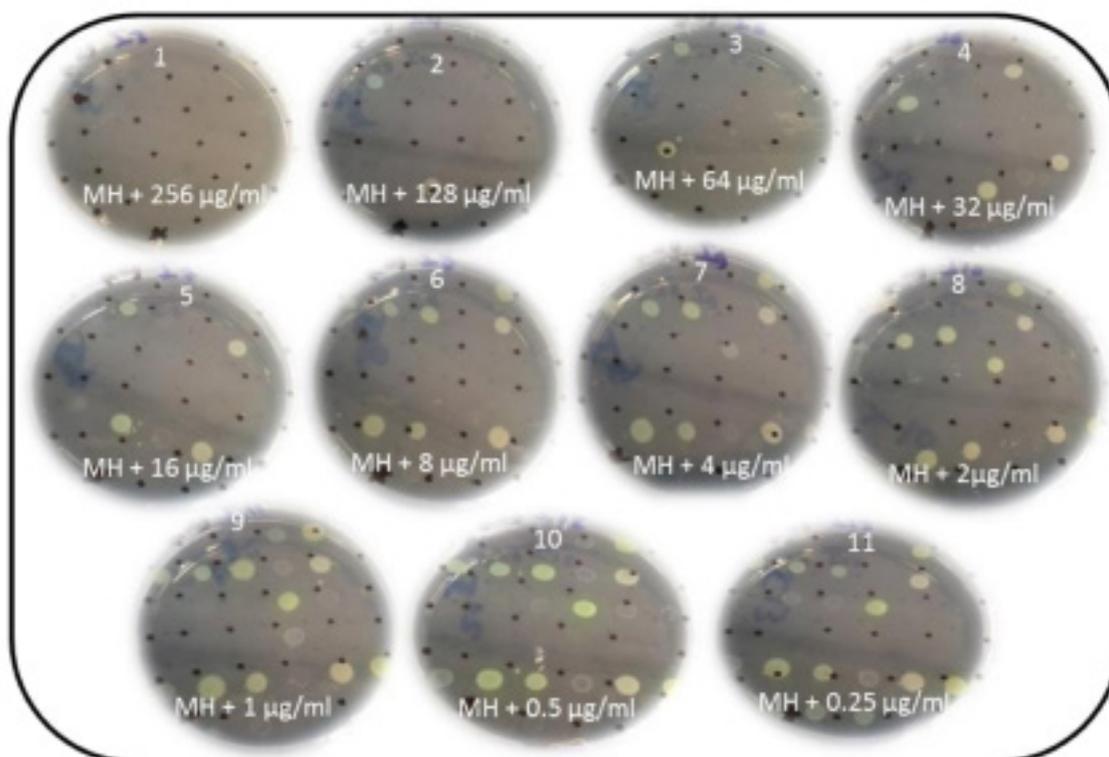


Figure 2: *P. aeruginosa* MIC against Imipenem antibiotic

Table (1): Minimal inhibitory concentration (MIC) of Imipenem and Meropenem against *P. aeruginosa* isolates.

MIC of antimicrobial drugs					
Isolate's Number	IMP (µg/ml)	MEM (µg/ml)	Isolate's Number	IMP (µg/ml)	MEM (µg/ml)
7	8	16	99	8	4
15	2	2	102	64	32
29	2	32	103	16	128
37	4	8	107	64	4
50	256	32	108	2	8
51	4	32	109	16	16
52	64	8	110	32	16
53	2	4	111	16	16
56	8	32	112	16	16
65	16	32	114	64	8
73	512	512	115	32	4
79	2	2	116	32	4
80	2	4	117	8	8
81	16	128	120	64	4
86	4	16	123	2	4
87	64	64	124	8	16
94	128	512	125	2	32
96	16	32	126	1	8
97	4	8	147	8	64
98	8	16			

IMP: (Imipenem). MEM: (Meropenem).

MIC of 2µg/ml, 8 isolates had an MIC of 4µg/ml, 7 isolates had an MIC of 8µg/ml, 8 isolates had an MIC of 16µg/ml, 8 isolates had an MIC of 32µg/ml, 2 isolates had an MIC of 64µg/ml, and 2 isolates for each had MIC 128µg/ml, and of 512µg/ml. there were a variety of difference from study completed by Hemalatha *et al* that observed MIC ranges from 8-128 µg/ml (Hemalatha et al., 2005).

4. Conclusion

P. aeruginosa are quiet critical micro-organism in the treatment therapy; as the resistance rate is increasing in response to illegal antimicrobial use especially in the developing counties. API 20NE provide a rapid and accurate species identification. Antibiotic have been monitored and rotated on hospital use to avoid

bacterial resistance.

5. Conflict of interest

The authors report no declaration of conflict of interest.

6. References

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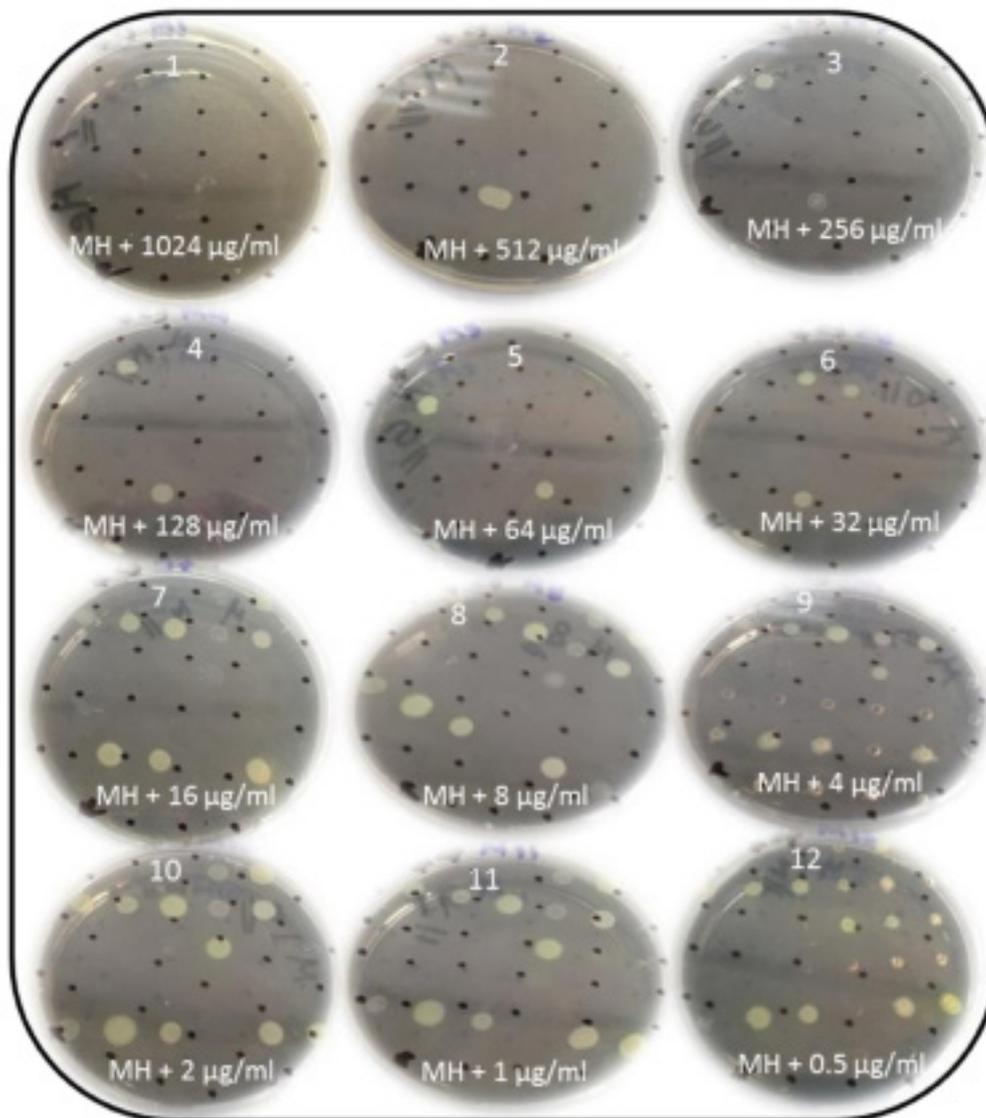


Figure 3: *P. aeruginosa* MIC against Meropenem antibiotic

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