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Antibiotic susceptibility patterns of local strains of Pseudomonas aeruginosa

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ABSTRACT: The resistance patterns of 100 local strains of *Ps. aeruginosa* were investigated using two different methods: (1) Broth Dilution and (2) ε-test. From the seven antibiotics tested, *Ps. aeruginosa* showed a 100% sensitivity to imipenem (n=30). Among the first-line agents, azlocillin, ceftazidime, and gentamicin showed the highest sensitivity rates, 87%, 93% and 92%, respectively. Aztreonam and ciprofloxacin showed the presence of intermediately sensitive strains, with 61% of the isolates tested being fully sensitive to each antibiotic. Only 47% of the strains were found to be sensitive to ceftriaxone. The results obtained were similar to studies carried out abroad.

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Introduction

Ps. aeruginosa can colonise healthy individuals as a harmless saprophyte. It is known to be a major cause of nosocomial infections particularly as it grows virtually everywhere including respirators used in operating theatres. It is one of the most important hospital pathogens especially in patients with a degree of immunosuppression. It is important because many strains show a degree of multiple drug resistance. The degree of resistance to several antibiotics among clinical isolates of *Ps. aeruginosa* has been increasing in recent years.

Bronchopneumonia, septicaemia, meningitis, and urinary tract infections caused by *Ps. aeruginosa* may be acute or chronic in relation to the host immune reactivity. *Ps. aeruginosa* is also a cause of severe epidemic diarrhoea in infants, ocular and burn infections, osteomyelitis and malignant external otitis.

The pathology of a *Ps. aeruginosa* infection includes necrosis and haemorrhagic lesions, infiltration by mononuclear cells, cytotoxic effects on macrophages and polymorphonuclear leukocytes, and widespread granulomatous lesions.

According to the National Nosocomial Infections Surveillance System (NNIS), *Ps. aeruginosa* is the third most important nosocomial pathogen (11%)[1].

Methods

The samples used in this study were taken from clinical isolates which were identified as *Ps. aeruginosa* by the laboratory staff as part of their routine work.

(1) The Broth Dilution Technique

Iso-Sensitest Broth® was used for this method. Each sample that did not show growth was inoculated on *Blood Agar* to determine whether a particular concentration was inhibitory or bactericidal. The lowest concentration (i.e., highest dilution) which did not show growth on the agar plate was the MBC. By determining both MIC and MBC tolerance was also estimated. Tolerance is of the utmost importance in cases where bactericidal therapy is a requirement.

(2) ε-Test technique

Half the plate was swabbed with the control strain (ATCC 27853), the other half being inoculated with the test strain so that in using this procedure each ε-test strip had a simultaneous control run[2].

ε-Test MIC values have been shown to be reproducible and directly proportional to MIC values from the NCCLS reference agar dilution procedure[3].

Results

The MIC50 and MIC90 data together with the MIC range for the seven antibiotics tested are shown in Table 1.

Figure 1 shows the qualitative classification of the data obtained. Isolates are classified as Sensitive, Intermediate or Resistant to the respective antibiotic. The breakpoints followed were those recommended by the European Committee for Clinical Laboratory Standards (ECCLS).

Tolerance

Tolerance is when the MIC/MBC ratio is 32 (i.e. the difference between MIC and MBC is ≥ 6 two-fold dilution factors. A high frequency of tolerance was found with azlocillin, as illustrated in Figure 2.

Table 1 - In vitro activity of antibacterial agents against *Ps. aeruginosa*.

Antimicrobial Agent	MIC50 (µg/ml)	MIC90 (µg/ml)	Mean MIC (µg/ml)	MIC range (µg/ml)	Breakpoints	Percent Susceptible
Azlocillin	4	128	13.64	<8 - >256	S= <64 R= >128	87
Aztreonam	8	32	6.68	<2 - >64	S= <8 R= >32	81
Ceftazidime	2	8	3.03	<2 - >64	S= <16 R= >32	97
Ceftriaxone	64	64	24.06	2 - >32	S= <16 R= >32	47
Ciprofloxacin	1	16	1.04	<0.25 - >8	S= <1 R= >4	79
Gentamicin	2	8	2.69	<1 - >32	S= <4 R= >8	92
Imipenem	1	2	1.17	0.25 - 4	S= <4 R= >16	100

Fig. 1 - Qualitative Results

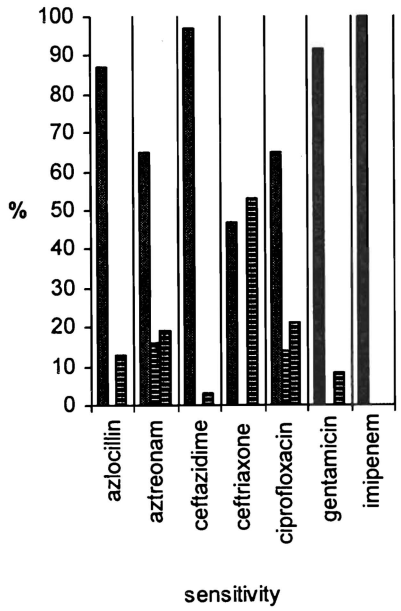
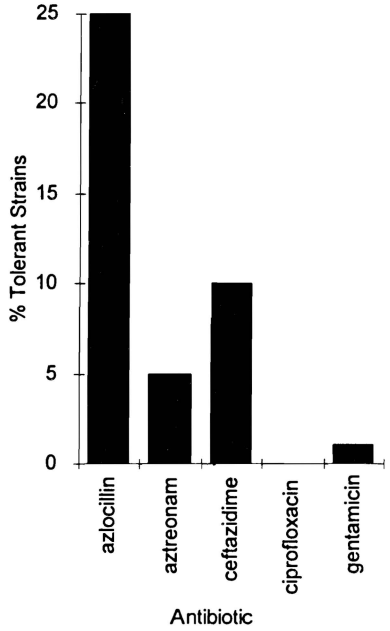


Fig. 2 - Tolerance



Multiple Drug Resistance

Multiple Drug Resistance (MDR) is yet another distressing problem. Figure 3 shows the resistance patterns of the 100 isolates studied. All the strains were fully sensitive to imipenem.

The criterion chosen for defining Multiple Drug Resistance was that an isolate results resistant to two or more antimicrobial agents excluding ceftriaxone which is not an antipseudomonal cephalosporin. Thus a total of 14 isolates were classified as MDR. Figure 3 shows the resistance patterns of the 100 isolates studied.

Comparison with other studies

Table 2 summarises the comparison of results obtained in this study with studies carried out abroad. The results obtained in different studies follow the same trend in resistance and any differences in results are mainly due to geographical variation in resistance patterns. The only relevant difference is with ciprofloxacin to which Maltese strains show higher levels of resistance.

Discussion

Tolerance

If a species has high resistance to an antibiotic (e.g., ceftriaxone, Figure 1), this can be detected by routine

Fig. 3 - Multiple Drug Resistance

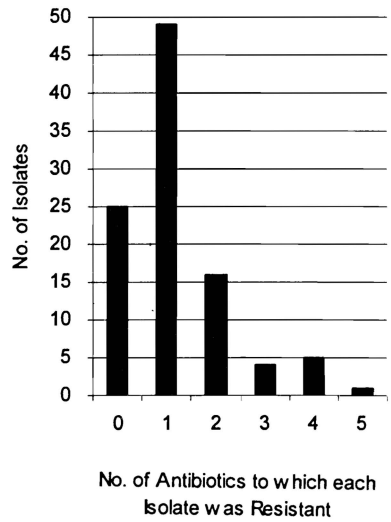


Table 2. Comparison with other studies.

Antibiotic	Sensitivity (%) Malta	Abroad	Nation	Author and year
Azlocillin	87	62	Germany	Ansorg, et al. , 1990[4]
Aztreonam	81	78.3	Multicentre study	Verbist, 1994[5]
		88.0	Germany	Abb, 1992[6]
		90.4	Greece	Sofianu, et al , 1989[7]
Ceftazidime	97	91.7	Multicentre study	Verbist, 1994[5]
		93.0	Germany	Abb, 1992[6]
		82	Germany	Ansorg, et al., 1990[4]
		93.0	Greece	Sofianu, et al, 1989[7]
Ceftriaxone	47	<50	Germany	Ansorg, et al., 1990[4]
		62.0	Greece	Sofianu, et al., 1989[7]
Ciprofloxacin	79	90.0	Germany	Abb, 1992[6]
		90	Germany	Ansorg, et al., 1990[4]
Gentamicin	92	84.0	Germany	Abb, 1992[6]
		38.4	Greece	Sofianu, et al., 1989[7]
Imipenem	100	93.5	Germany	Abb, 1992[6]
		91	Germany	Ansorg, et al., 1990[4]
		99.5	Greece	Sofianu, et al., 1989[7]

laboratory investigations. On the other hand, tolerance is only detected if actual MICs and MBCs are measured. Thus, an antibiotic such as azlocillin seems to be active against *Ps. aeruginosa* (Figure 1), but in practice, many number of strains are actually tolerant to it (Figure 2). This is important in patients who require bactericidal drugs.

Multiple Drug Resistance

All three isolates which were resistant to ceftazidime showed cross resistance with ceftriaxone. However, four MDR isolates were sensitive to ceftriaxone and resistant to unrelated agents. This is important since only 47% of the isolates were sensitive to ceftriaxone. Thus, there is still a place for the use of ceftriaxone as an antipseudomonal agent even though more than 50% of isolates are resistant.

Conclusions

Ps. aeruginosa is one of the most important pathogens in hospitals, accounting for 11% of all hospital acquired infections, according to the National Nosocomial Infections Surveillance System, 1989[1]. Therefore, its resistance to a number of antimicrobial agents poses problems in the empiric selection of an appropriate drug for the treatment of infections caused by this organism.

According to the results obtained, ceftazidime and gentamicin should be the empirical first line drugs to use against *Ps. aeruginosa* infections since these two antibiotics are the only ones with a percentage sensitivity greater than 90. The second line agent imipenem should be reserved for strains resistant to first line agents, especially those resistant to both gentamicin and ceftazidime.

Table 3. Health Service cost (Lm) of parenteral therapy

Antibiotic	Dose/phial	Cost/phial	Dose	Regimen	Cost for 7 days
Azlocillin	2.0g	1.94	2.0g	t.d.s.	40.74
Aztreonam	500mg	1.62	1.0g	t.d.s.	68.04
Ceftazidime	1.0g	2.90	1.0g	t.d.s.	60.90
Ceftriaxone	2.0g	10.00	2.0g	b.d.	140.00
Ciprofloxacin	200mg	8.77	200mg	b.d.	122.78
Gentamicin	40mg/ml	0.62	80mg	t.d.s.	13.02
Imipenem	500mg	8.40	500mg	t.d.s.	176.40

When choosing the right antibiotic the price factor should be taken into account. Table 3 shows the approximate cost of treatment for a week with the seven antibiotics tested in this study. From this table one can see that gentamicin is the cheapest antibiotic whereas imipenem is the most expensive. This may be one reason why gentamicin is used as a first line agent even though it has a number of potential side effects and precautions for use, whereas, the safer antibiotic imipenem is classified as a second line agent. However, the most interesting finding was obtained by comparing resistance levels to ciprofloxacin in Malta with two studies carried out in Germany. In Malta only 79% of the isolates were sensitive to ciprofloxacin as opposed to 90% in both German studies. This suggests the overuse or misuse of ciprofloxacin in Malta. It is likely to happen with any antibiotic which can be given by the oral route. In such a case, as with ciprofloxacin, there can be overuse of the antibiotic both in hospital and especially in community practice. A study of the development of resistance by *Ps. aeruginosa* during therapy with ciprofloxacin was published in 1989[8]. This is even more alarming when considering that ciprofloxacin can induce low level resistance to structurally unrelated antibiotics in Ps. aeruginosa and methicillin resistant *Staphylococcus aureus*[9].

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