Sensitivity Pattern and Beta-Lactamase Production in Clinical Isolates of Aeromonas Strains

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Altaf Ahmed,Saleem Hafiz (Ziauddin Medical University and Karachi.)
Qazi Touseef Ahmed,Hina Majeed,Serajuddaula Syed (Medical and Dental CoIlege, Karachi.)

Abstract

Of the 43 Aeromonas spp. isolated from various clinical samples 94% isolates were Beta-lactamase producers. The isolates were tested for sensitivity by disc diffusion method which is commonly used in Pakistan. MIC was determined by using Epsilometer test (E-test) method. More than 80% isolates were sensitive to cephalosporins and quinolones. However, resistance to commonly used antibiotics was very high, 94% isolates were resistant to ampicillin which corresponds to the betalactamase prodkction. More than 60% of the isolates were resistant to cotrimoxazole and 40% to chioramphenicol, hence quinolones and cephalosporins appear to be the drugs of choice for treating serious Aeromonas infections. The MIC range of Aeromonas was best for cefotraxime <0.06- 1.0 ug/mI. MIC 90 for cefotaxime was 0.50 ug/ml, for imipenem 0.25 ug/ml and for ciprofloxacin 2.0 ug/mI (JPMA 48:158, 1998).

Introduction

Aeromonas is widely distributed in nature. It has been found in fresh waters (rivers and lakes), sewage and food[1,2]. They are the causative agents of intestinal and extraintestinal infections in humans[3]. Extraintestinal infections include bacteraemia[4,5] and wound infections[6-8] mainly. Aeromonas bacteraemia is associated with haematological malignancies, solid tumors, hepato-biliary dysfunction (particularly cirrhosis) and trauma[3-5]. Intestinal infection presents itself in different ways. It may vary from acute, profusely watery diarrhoea with fever and vomiting to dysentery-like illness with cramps and abdominal pain. It sometimes gives rise to mild chronic diarrhoea which may last from 10 days to several months[3].

Knowledge of susceptibility patterns of local aemmonas strains is important, so that medical practitioners know the likelihood of activity of various antibiotics for empirical therapy of suspected aeromonas infection. MIC data for Aemmonas species in Pakistan has not been reported before: The current study is focused on the susceptibilities of Aeromonas spp. isolates from diarrhoeal samples, blood samples and other sites to various antibiotics in this region. MIC data for Aeromonas is also included in this study.

Material and Method

Forty three Aeromonas spp. were isolated from patients infected with Aeromonas spp. ranging from septicaemia, gastrointestinal and wound infections. These organisms were isolated on MacConkeys agar No.3 (Oxoid-CM115) and Ampicillin sheep blood agar (ASBA) (Muller-hinton agar-Oxoid-CM3337 +5% Sheep blood + Ampicillin selective supplement SR 136)[9].

All isolates were stored in 10% glycerol broth at -20°C. Sensitivity and MIC tests were performed in one batch. When needed broth were thawed and inoculated onto blood agar plates, incubated over night, checked for purity and results were confirmed by API 20 NE (Analytab Products tlnemational, S.A. Vercieu, France).

Sensitivity tests were done on Iso sensi agar (Oxoid) with commercial discs (Oxoid) according to the method of Kitby and Bauer[10] to the following antibiotics: Imipenem (10 ug), Mempenam(10 ug), Furazolidone (50 ug), Ofloxacin(10 ug), Chloramphenicol (30 ug), Ampicillin, (25 ug), Ceftriaxone (30 ug), Tetracycline (30 ug), Fosfomycin (50 ug), Cefotaxime (30 ug), Cefuroxime (30 ug), Ceftazidime (30 ug), Cefexime(5 ug), Aztreonam(30 ug), Tobramycin(10 ug), Gentamicin, (30 ug), Amikacin (30 ug), Sisomycin (10 ug); Cefaclor (30 ug), Amox-clav (30 ug), Co-Trimoxazole (25 ug), Nalidixic acid (30 ug).

Beta lactamase test

All isolates were tested forbeta lactamase production by using Chromogenic substract test:

One milligram Nitrocefin vial (Glaxo-SR 112), was reconstitued by adding 2 ml of rehydration fluid (Glaxo-SR112A), which gave a working solution of 500ug/ml. WhatmanNo. 1 filterpaperdisc was taken inapetri dish and impregnated with this solution.

One well isolates Aermonas colony was picked up from blood agar plate and applied on the paper with a wooden stick; A positive and negative control was put up with the test. A brick red colour developing within 15 minutes confimied the presence of beta- Iactamase[11].

Epsilometer test (E-Test) for the determination of MIC's

The Minimum Inhibitory Concentration (MIC) were performed by epsilometer test (AB Biodisk, Solna Sweden). This is a in-vitro susceptibility testing method designed for quantitative determination of susceptibility to antimicrobial agents. The test is effective for both non fastidious Gram negative and Gram positive aerobic bacteria, such as Enterobacteriaeceae, Pseudomonas, Staphylococcus and Enterococcus and fastidious bacteria, such as anaerobes, Pneumonococcus and Haemophilus. The system comprises a pre-defined antibiotic gradient on one side of a 50 mm long and 5 mm broad plastic strip. The MIC interpretive scale corresponds to 15 two fold MIC dilutions on other side of scale. These were used to determine the MIC in ug/mi of individual antibiotics against bacteria as tested on agar media by overnight incubation[12-16].

The test was performed by emulsifying a few well isolated colonies of Aeromonas in thioglycolate broth and compared to 0.5 McFarland. A sterile swab was dipped into the inoculum and the entire sensitest agar plate surface was swabbed aseptically and dried in incubator prior to the application of the E test strips. The strips were placed carefully and aseptically on the agar surface with the help of a sterile forceps ma way that the MIC scale was facing upwards and the maximum nearest the periphery of the plate (Figure 1).

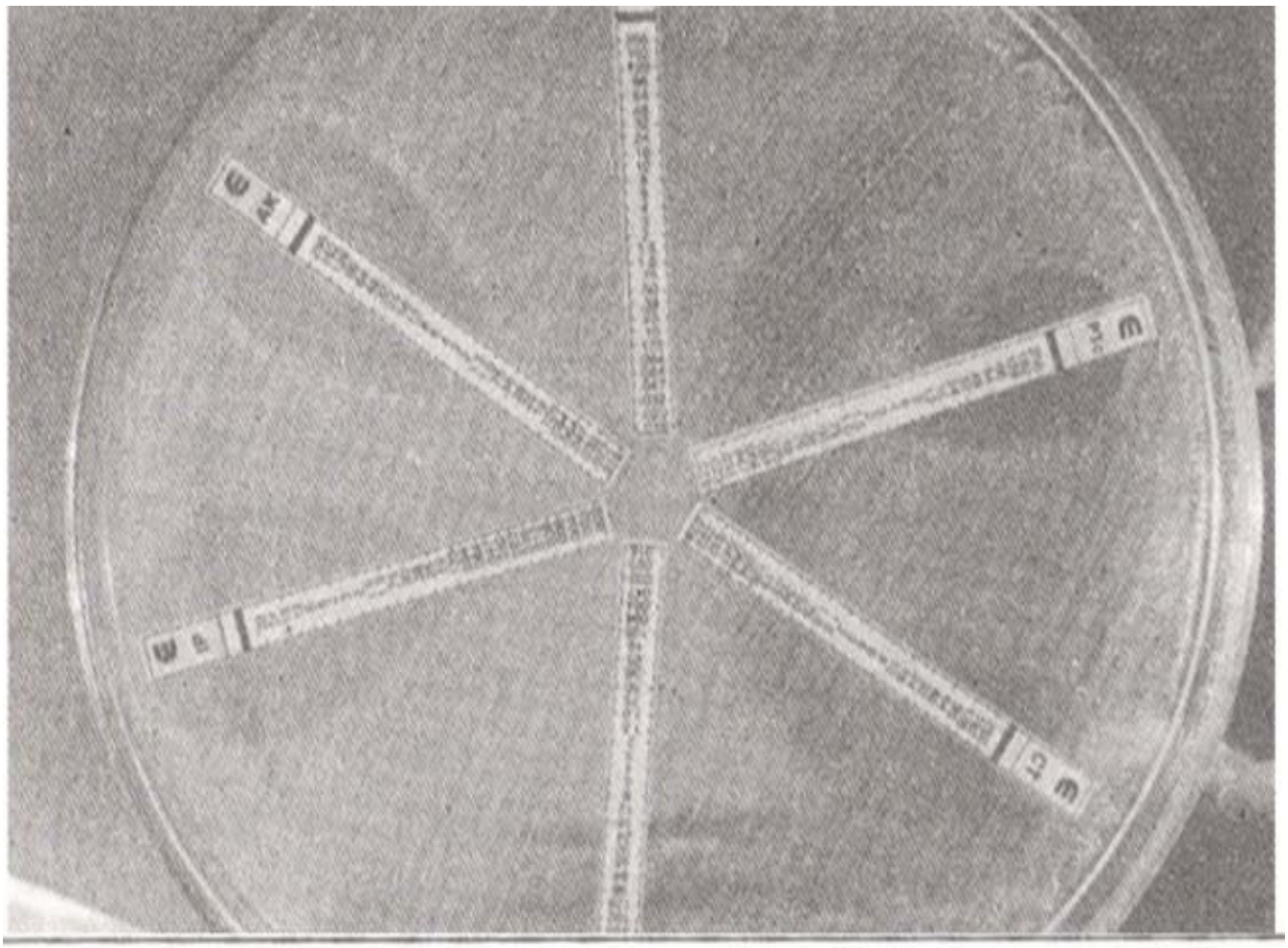

Figure 1: E-test demonstrated with an Aeromonas strain. Antibiotic abbreviations on the E-test strips and MIC interpretations are IP (imipenem 0.25 ug/ml), AK (amikacin, 2 ug/ml), CT (Cefotaxime, 32 ug/ml)

The plate was incubated at 35°C for 16-18 hours. The MIC value was read at the point of intersecion between the zone edge and the E test strip (Figure 2).

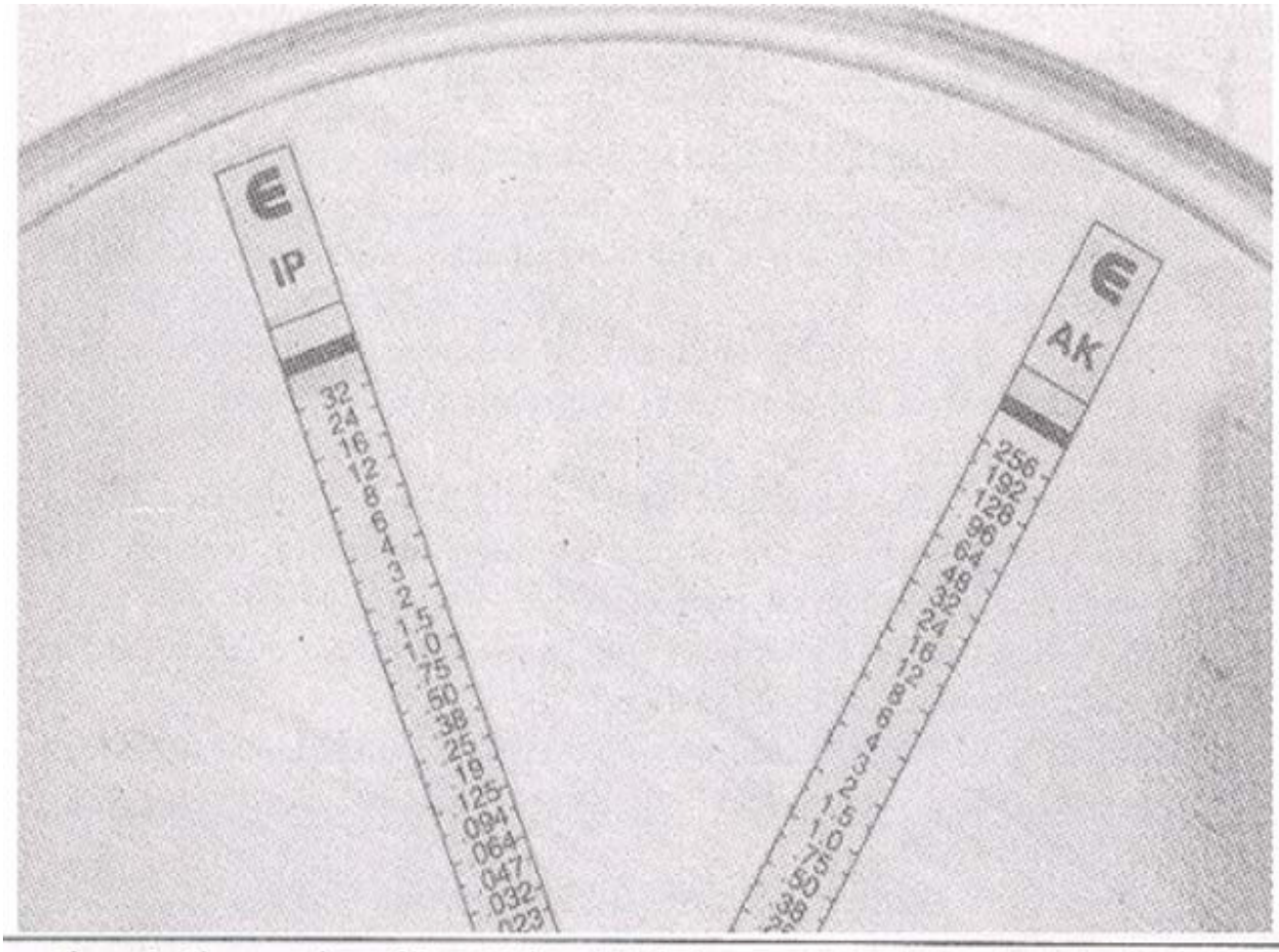

Figure 2. Close up view of E-test strips of IP (imipenem; 0.25 ug/ml) and AK (amikacin; 1.5 ug/ml) demonstrating the point at which the zone of inhibiton of bacterial growth intersects the antimicrobial strips.

Aeromonas hydrophila- ATCC 7966, Aeromonas caviae - ATCC 15468, Eschenchia coli -ATCC 25922 were used as controls.

Results

Ninety-four percent isolates of Aemmonas were betalactamase producers confirmed by chromogenic substrate test. Table I records the results of sensitivity pattern of Aeromonas to the commonly used antibiotics. More than 80% isolates were sensitive to cefixime, ofloxacin, ceftriaxone and furazolidone. While only 6.3% isolates were found to be sensitive to ampicillin and 23.3%to cotrimoxazole whicitare the most commonly used antibiotics. Sensitivity to other commonly used agents ranged from 40% to 100% based on discs sensitivity testing.

Table H records the results of Minimum Inhibitory concentration 50 and 90 of Aeromonas E-test. This method is most currently used for determining the MIC of various antibiotics.

Table I. Sensitivity pattern of aeromonas species.

	Antibiotics	Total Samples	Sensitive No.	Sensitive %
1.	AMP	43	2	6.3
2.	AUG	43	17	39.5
3.	CEC	43	30	69.8
4.	CRO	43	35	81.4
5.	CXM	43	28	65.1
6.	CAZ	43	37	86.0
7.	CTX	43	37	86.0
8.	CFM	38	31	81.6
9.	AZT	43	41	95.3
10.	IMP	43	42	97.7
11.	MEM	43	43	100 .0
12.	CN	43	38	88.4
13.	TOB	43	37	86 .0
14.	AK	43	37	86 .0
15.	SIS	43	30	69.8
16.	TET	43	33	76.7
17.	SXT	43	10	23.3
18.	OFX	43	37	86.0
19.	CL	43	27	62.8
20.	FOS	43	33	76.7
21.	FR	43	35	81.4
22.	NA	43	24	55.8

Abbreviations: AMP, Ampicillin; AUG, CoAmoxclav; CEC, Cefaclor; CRO, Ceftriaxone; CXM, Cefuroxime; CAZ, Ceftazidime; CTX, Cefotaxime; CFM; Cefexime; AZT, Aztreonam; IMP, Imipenem; MEM,Meropenem; CN, Gentamicin; TOB, Tobramycin; AK, Amikacin; SIS, Sisomycin; TET, Tetracycline; SXT, Co-trimoxazole; OFX, Ofloxacin; CL, Chloramphenicol; FOS, Fosfomycin; FR, Furazolidone; NA,Nalidixic acid.

The table shows MIC to various antibiotics ranging from 0.06 ug to 256 ug among them MIC 90 for Imipenem was 0.25 .ug, cefotaxime 0.5 ug, cipmfloxacin and gentamycin 2.0 ug, amikacin and cefatazidime 4.0 ug ceftriaxone and cefuroxime 32 ug and to aztreonam and co-amox/clav 64 ug.

Table II. The MIC 50 and 90 of Aermonas spp. to antibiotics based on "E-test" and their range.

Antibiotic	MIC 50	MIC 90	Range
Imipenem	0.13	0.25	<0.06-4.0
Ceftazidime	0.50	4.0	0.13-128
Ceftriaxone	0.13	32	<0.06-28
Cefotaxime	0.25	0.50	<0.06-1.0
Cefuroxime	4.0	32	0.25-256
Gentamicin	1.0	2.0	<0.06-16
Amikacin	1.0	4.0	0.25-16
Aztreonam	0.13	64	<0.06-56
Ciprofloxacin	0.13	2.0	<0.06-56
Co-Amox/Clav	8.0	64	<0.06-64

MIC values play an important role in determining the fate of the organism isolated from different site as variable degree of levels are achieved at different sites, hence now MIC value have become very important.

Discussion

Local isoates of Aennonas spp. showed higher rate of resistance to ampicillinwhich was expected, andahighdegree of resistance to cotritnoxazole in contrast to the findings by others[17,18]. This is because co-trimoxazole is one of the most frequently prescribed drugs by general practitioners in the region. Overall aeromonas isolates have shown a higher degree of resistance to almost all antibiotics which may be due to their misuse by the doctos and quacks.

Ciprofioxacin is a good choice as it is available orally and more than 80% isolates were sensitive. However, care should be taken not to use it indiscriminately and should notbe prescribed for children under 10 years of age. Our results confirmed the results of others that chlorampbenicol and ciprofloxacin are consistantly active against Aeromonas spp.[19-21]. Tetracycline was also active although resistance rates of 20% were seen. Morn than 90% of the Aeromonas strains were found to be sensitive to Aztreonam, Imipenem and Meropenem. These antibiotics should not be used routinely and must be reserved for very serious and resistant cases. Aminoglycoside have similar activity against Aeromonas. The MIC 90 to Aztreonam, cefthaxone and cefuroxime is higher in our study as compared to other studies[20-24]. This could be due to the fact that Aztreonam and ceftnaxone are frequently used in Pakistan.

Ninety-four percent of the isolates were beta-lacatamase producers in this study. Chrosomally-encoded, induciblebeta-lactamasesactivity has been associated with resistance to a wide range of extended spectrum penicillins, cephalosporins, monobactams and carbapenems in cincal isolates of Aemmonas spp[23], identified several beta-lactamases mA. hydmphilia, A. sobna and A. caviae. Plasmid-mediated

resistance does not appearto be a problem in Aeromonas spp. [25]
The MIC was performed by using the new Epsilometer test (E test). It was found to be accurate, time saving and less laborious method for MIC testing with minimum chance of interpretation error. The test was technically very simple and needed no special equipment and the methodology was familiar, resembling agar disk diffusion method.
E test MIC values have been shown to be reproducible and directly proportional to MIC values from the National Committee for Clinical Laboratory Standards (NCCLS) reference agar dilution procedure[14-16]. TheEtest has proven to be an excellent addition to the list of methods available for antimicrobial susceptibility testing in this study. Considering the cost of this method itis obvious that this method will notbe very economical, especially when testing large number of drugs on numerous isolates, but it is avery convenient method which can be used in difficult to treat cases.
Gastrointestinal infections with Aemmonas spp. are generally sell-limiting. It is well known that children who are prescribed antibiotics during the course of diarrhoea were more likely to suffer from persistent diarrhoea than children who did not[26-29]. Thus antimicrobial therapy is indicated only in patients with diarrhoea who have high fever and systemic toxicity, dysenteric disease or travelers diarrhoea[30] or systemic infection in immunocompromised patient. But Aeromonas from any other source could be life threatening and should be treated with appropriate antimicrobial agent.
It is concluded that empirical reasonable choices for serious infections like septicaemia, are certain cephalosporins (cefotaxime, ceftriaxone and ceftazidine), aminoglycosides (gentamicin, tobramycin and amikacin), ciprofloxacin and aztreonam rather than ampicillin or co-thmoxazole. Use of Monobactams (imipenem and meropenem) should be restricted as third line drugs.

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