Antibiotic susceptibility profile of *Aeromonas* spp. isolates from food in Abu Dhabi, United Arab Emirates

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SUMMARY

A total of 57 *Aeromonas* isolates from food samples such as fresh and frozen chicken, game birds, pasteurized milk, baby food, bakery products, fruit and vegetables, fish, and water from Abu Dahbi, UAE were investigated for antibiotic susceptibility profile. Most strains were resistant to penicillins (ticarcillin, mezlocillin, oxacillin, piperacillin), sulfamethoxazole, trimethoprim and macrolides (erythromycin, vancomycin, clindamycin) but sensitive to tetracycline, chloramphenicol, nitrofurantoin, aminoglycosides (amikacin, gentamicin, tobramycin), cephalosporins (cefuroxime, ceftrioxone, cefazolin, cephalexin, cephalothin, cefoxitin, cefotaxime), quinolone (ciprofloxacin), colistin sulphate and SXT (trimethoprim-sulfamethoxazole). On the other hand, many antibiotics showed excellent inhibitory activity (>75% strains were sensitive to them) against all the strains tested. These include cefuroxime, ceftrioxone, ciprofloxacin, colistin, amikacin, gentamicin, tetracycline, chloramphenicol, nitrofurantoin, cefotaxime and tobramycin. In conclusion, the results show a detailed pattern of sensitivity of the various *Aeromonas* spp. isolates to a variety of antibiotics and provide useful information in the context of selective isolation and phenotypic identification of the aeromonads from food.

KEY WORDS: Aeromonas spp. antibiotic susceptibility profile, Food, Abu Dhabi, United Arab Emirates

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INTRODUCTION

The aeromonads are ubiquitous in aquatic environments and they are frequently isolated from different foods and drinking water (Baloda *et al*., 1995; Gavrie *et al*., 1998; Hudson and De Lacey, 1991; Krovacek *et al*., 1992). *Aeromonas* spp. have been reported to be involved in a wide spectrum of diseases in humans and animals (Carnahan *et al*., 1991; Krovacek *et al*., 1994; Misra *et al*., 1989; Pasquale *et al*., 1994).

The antibiotic sensitivity of an isolate is usually required for effective clinical control, especially when it is from a clinical specimen. Tetracycline and oxytetracycline are usually used to treat the infections (De Paola *et al.,* 1998). Antibiotic-susceptibility pattern is also important for selective isolation of microorganisms. The aeromonads have been regarded as universally resistant to penicillins (penicillin, ampicillin, carbenecillin, and ticarcillin) for quite a long time. For this reason, ampicillin has been generally incorporated in the culture media for selective isolation of the aeromonads from contaminated samples.

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However, owing to the discovery of ampicillin-sensitive strains of the aeromonads (Carnahan *et al*., 1991; Shanon *et al.,*1986) its use is being abandoned. Carnahan *et al*. (1991) stated that the ampicillin-containing media may result in a negative selection against *Aeromonas trota* which has been in the environment for quite some time and appears to be capable of causing human disease. In addition, this awareness of ampicillin-susceptibility is not only important for *A. trota* but also for other aeromonads because ampicillin-susceptible strains of *A. caviae* have also been recovered from clinical specimens. Misra *et al.* (1989) related the lower recovery of *A. sobria* on Butzler Campylobacter selective agar to the concentration of cefazolin and colistin present in it, which may inhibit the growth of the aeromonads. They concluded that the Campylobacter selective medium with Skirrow or Blaser antimicrobial supplement is not suitable for Aeromonas isolation since most of the Aeromonas strains are susceptible to trimethoprim and polymyxin B.

In addition, Janda and Motyl (1985) proposed that cephalothin susceptibility could be a potential phenotypic marker in the identification of *Aeromonas sobria*. Carnahan *et al.* (1991c) evaluated the susceptibility of the various Aeromonas species to cephalothin and incorporated it in their identification scheme, the Aerokey-II. *A. hydrophila* and *A. caviae* are often more resistant to cephalothin than is *A. sobria* (Motyl *et al*., 1985).

Resistance of the aeromonads to penicillins and other antibiotics has been explained to be due to presence of plasmids. DePaola *et al.* (1988) stated that *A. hydrophila* acquires resistance to commonly used antibiotics through transfer of R-factors. The occurrence of plasmids resistant to ß-lactam antibiotics and other drugs in the aeromonads has also been described (Aoki *et al.,* 1971; Chaudhuri *et al.,* 1996). The resistance markers harbored on such elements include those for tetracycline, ampicillin, tobramycin and kanamicin (Janda 1991).

The resistance of the aeromonads to ampicillin and related drugs is due to the presence of at least four β-lactamases (von Graevenitz and Altwegg 1991; Shanon *et al.,* 1986; Sykes and Mathew 1976). This is why Carnahan *et al.* (1991) recommended the Bauer-Kirby agar disc diffusion method for the determination of susceptibility to cephalothin since the aeromonads have inducible β-lactamases.

In addition, most of Aeromonas species are susceptible to aminoglycosides, chloramphenicol, tetracycline, trimethoprim-sulfamethoxazole, and quinolones (Motyl *et al.,* 1985; Renhardt and George 1985). They are also susceptible to azlocillin, piperacillin and the second and third generation of cephalosporins (von Graevenitz and Altwegg 1991). *A. jandaei* is resistant to cephalothin, colistin, cefazolin, cephalothin, imipenem, piperacillin and ticarcillin (Carnahan *et al*., 1991).

The present study reports the antibiotic susceptibility patterns of *Aeromonas* spp. isolated from different food and environmental samples in Abu Dhabi, United Arab Emirates.

MATERIALS AND METHODS

Samples

A total of 57 *Aeromonas* isolates from fresh and frozen chicken, game birds, pasteurized milk, baby food, bakery products, fruit and vegetables, fish, water from Abu Dahbi, UAE were investigated for antibiotic susceptibility profile (Tables 1 and 2).

Isolation and biotyping of Aeromonas spp

Twenty five grams of respective food samples were homogenized (if required) in 225 ml of peptone water. Tenfold serial dilutions were made and 0.1ml amounts were plated on Difco *Aeromonas* medium (BD, Becton, Dickinson and Company Sparks, MD 21152, USA) and Oxoid *Aeromonas* ampicillin medium (Oxoid, CM 833, SR136). The agar plates were incubated at 30°C for 24 h.

The material from these enrichment samples was transferred with a loop on the above two media and the samples were incubated under similar growth conditions.

The presumptive *Aeromonas* colonies were streaked on 5% (v/v) blodagar plates (BD) containing washed horse erythrocytes. Only those isolates, which were Gram-negative rods, motile, oxidase-positive, glucose fermenting, O/129 resistant isolates were considered aeromonads (2). The isolates were further investigated by bio-

chemical typing using API 20 NE (API 20NE, Analytab Products, Marcy-l'Etoile, France) and by using Aerokey II (2). Pure isolates were frozen in brain heart infusion broth (BD) with 15% glycerol and stored until tested.

Antibiotic susceptibility test

The *Aeromonas* spp. strains were kept on nutrient agar slants at room temperature and maintained by subculturing every month. Susceptibility of these strains was determined using MASTRING-S (Mast Diagnostics, Mast Laboratories Ltd. UK). It comprised ampicillin (10 µg), ticarcillin (75 µg), gentamicin (10 µg), cephalothin (30 µg), trimethoprim (1.25 µg), sulfamethoxazole (25 µg), tetracycline (10 µg) and colistin sulphate (25 µg) discs. Mueller-Hinton agar plates were inoculated using a swab of 4-hour trypticase soy broth culture of the test isolate. After drying the surface, the ring was placed on the agar surface using sterile forceps. The plates were incubated at 37°C for 24 hours. They were examined for zone of inhibition, measured in millimeters using a measuring scale. The susceptibility interpretation was made according to the manufacturer's instructions. Susceptibility to the rest of the antibiotics/drugs (cefuroxime, Ceftrioxone, cefazolin, ciprofloxacin, cephalexin, mezlocillin, oxacillin, amikacin, clindamycin, erythromycin, chloramphenicol, vancomycin, sulfamethoxazole-trimethoprim (SXT), nitrofurantoin, cefoxitin, Cefotaxime, piperacillin, tobramycin) was determined using Sceptor® System MIC/ID panels i.e. enteric MIC/ID panel (80401) and Gram-Positive MIC/ID panel (80351) (Johnston Laboratories, MD). These panels were used and interpreted according to the manufacturer's instructions.

RESULTS

Table 1 shows the prevalence of *Aeromonas* spp. in different foods and environment in Abu Dhabi. The results of antibiotic susceptibility of the test strains for the various antibiotics and drugs are shown in Table 2. Most of the isolates were resistant to erythromycins (macrolides) but were sensitive to tetracycline, chloramphenicol, nitrofurantoin, quinolone and aminoglycosides. All the isolates were sensitive to colistin sulphate and gentamicin. The aminoglycosides (amikacin, gentamicin and tobramycin) showed excellent activity against almost all the isolates of the aeromonads except a few isolates of *A. caviae*. The most potent cephalosporins showing activity against most of the isolates of the major species (*A. hydrophila*, *A. caviae*, *A. veronii bv. sobria*) were cefuroxime, ceftrioxone and cefotaxime. The other four cephalosporins (cefazolin, cephalexin, cephalothin, cefoxitin) showed variable inhibitory activity against the various aeromonads but were most potent against *A. veronii bv. sobria*

TABLE 1 - *Prevalence of Aeromonas spp. in different food samples and environments in Abu Dhabi, United Arab Emirates.*

Aeromonas spp.	*Fresh chicken*	*Frozen chicken*	*Game birds*	*Milk*	*Bakery products*	*Fish*	*Water*	*Fruits/ Vegetables*	*Baby food*
A. hydrophila (11)	3	0	1	3[a]	1	1	2[b]	0	0
A. veronii bv. sobria (15)	7	1	0	2[a]	0	3	2[c]	0	0
A. caviae (25)	8	3	2		1	3	5[b]	2[e]	1
A. trota (3)	3								
A. schubertii (2)	1						1[b]		
A. jandaei (1)							1[d]		

[a]Pasteurized milk; [b]Well water; [c]Sea water; [d]Stored drinking water tank; [e]Frozen mixed vegetables.

TABLE 2 - *Antibiotic susceptibility of Aeromonas spp. isolated from Abu Dhabi, United Arab Emirates.*

Antibiotics	*Percent sensitive*						
	A. hydrophila	*A. veronii bv. sobria*	*A. caviae*	*A. trota*	*A.schubertii*	*A. jandaei*	*Cumulative*
Cefuroxime	72.7	93.3	65.4	66.7	100.0	100.0	75.9
Ceftrioxone	81.8	93.3	84.6	66.7	50.0	100.0	84.5
Cefazolin	27.3	86.7	15.4	33.3	50.0	0	37.9
Ciprofloxacin	90.9	100.0	88.0	66.7	100.0	100.0	89.6
Cephalexin	18.2	92.8	19.2	66.7	50.0	0	40.4
Trimethoprim	45.5	40.0	11.5	33.3	50.0	0	27.6
Sulfamethox.	9.1	0	3.8	0	0	0	3.4
Colistin sulphate	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Ticarcillin	0	40.0	53.8	33.3	0	0	36.2
Mezlocillin	9.1	0	23.1	0	0	0	12.1
Oxacillin	0	0	0	0	ND	ND	0
Cephalothin	28.6	92.3	28.6	66.7	50.0	ND	50.0
Amikacin	100.0	100.0	96.2	100.0	100.0	100.0	98.3
Gentamicin	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Clindamycin	0	0	5.5	0	ND	ND	3.0
Erythromycin	33.3	38.5	18.2	33.3	0	ND	26.5
Tetracycline	100.0	86.7	100.0	100.0	100.0	100.0	96.6
Chloram-phenicol	100.0	100.0	96.0	100.0	100.0	100.0	98.2
Vancomycin	0	0	0	0	ND	ND	0
Trimethoprim-sulfamethox.	90.9	53.3	46.2	100.0	50.0	100.0	60.3
Nitrofurantoin	100.0	100.0	96.2	100.0	100.0	100.0	98.2
Cefoxitin	33.3	100.0	52.2	100.0	100.0	100.0	66.7
Cefotaxime	90.9	100.0	96.0	100.0	100.0	100.0	96.5
Piperacillin	9.1	13.3	34.6	33.3	0	0	22.4
Tobramycin	100.0	100.0	96.2	100.0	100.0	100.0	98.3
Ampicillin	0	0	15.4	33.3	0	0	8.3

ND = not done

strains. Most of the strains of the aeromonads were resistant to acylureido-penicillin (piperacillin), though this antibiotic showed varying degrees of growth inhibition *in vitro*. Of the macrolides (erythromycin, vancomycin, clindamycin), only erythromycin showed some activity in nearly equal measure against the three major species. Almost all isolates were resistant to vancomycin and clindamycin. The quinolone ciprofloxacin showed excellent activity against the aeromonads tested in the present study. Sulfamethoxazole did not prove to be inhibitory against the aeromonads but when used in combination with trimethoprim, their activity increased significantly, especially against the *A. hydrophila* strains (SXT, Table 1). High inhibitory potential was observed in case of tetracycline, chloramphenicol and nitrofurantoin.

All the *A. trota* isolates were sensitive to colistin, amikacin, gentamicin, tetracycline, chloramphenicol, sulfamethoxazole-trimethoprim, cefoxitin, nitrofurantoin, cefotaxime and tobramycin. None of the strains was inhibited by sulfamethoxazole, mezlocillin, oxacillin, clindamycin and vancomycin. Both the studied strains of *A. schubertii* were sensitive to most of the antibiotics. These strains were resistant to sulfamethoxazole, ticarcillin, mezlocillin, erythromycin and piperacillin. One strain was resistant to cephalosporins. The single isolate of *A. jandaei* tested had a sensitivity pattern similar to that of *A. schubertii*. The cumulative sensitivity pattern of all the *Aeromonas* isolates is also shown in Table 2. Many antibiotics showed excellent inhibitory activity (>75% strains were sensitive to them) against all the strains tested. These include cefuroxime, ceftrioxone, ciprofloxacin, colistin, amikacin, gentamicin, tetracycline, chloramphenicol, nitrofurantoin, cefotaxime and tobramycin.

DISCUSSION

The results presented show a detailed pattern of sensitivity of the various *Aeromonas* isolates to a variety of antibiotics and drugs and provide useful information in the context of selective isolation and phenotypic identification of the aeromonads. The observations regarding the activity of the macrolides, tetracycline, chloramphenicol, nitrofurantoin, quinolone and aminoglycosides are comparable to the results obtained by several other investigators (Carnahan *et al*., 1991c; Carnahan *et al*., 1991; von Graevenitz *et al*., 1991; Pasquale *et al*., 1994). Rashad and Abdelkareem (1995) reported much higher resistance of the aeromonads for such antibiotics as colistin sulphate, amikacin, chloramphenicol, and tetracycline than demonstrated in the present work or by the other workers cited above. Also Petersen and Dalsgaard (2003), Komathi *et al*., (1998) and Ko *et al.,* (1996) found that most of their strains of Aeromonas are resistant to the commonly used antibiotics such as tetracycline, trimethoprim and chloramphenicol.

The *Aeromonas* species are resistant to penicillins in general but *A. trota* and some of the *A. caviae* strains are susceptible to them. The existence of penicillin-sensitive *A. caviae* has also been reported by Havelaar *et al.,* (1987) and Seidler *et al.,* (1989). Moreover, Motyl *et al.,* (1985) also showed that *A. caviae* is more susceptible to mezlocillin than the *A. hydrophila* and A. *veronii bv. sobria*.

The observation that all isolates of the aeromonads are sensitive to colistin sulphate and gentamicin received support from Hickman-Brenner *et al.,* (1987) and Seidler *et al.,* (1989). In contrast, Rashad and Abdelkareem (1995) found most of their strains to be resistant to these drugs. The sensitivity of all the isolates to colistin in the present study validates the suspicion of Misra *et al.* (1989) that the concentration of this drug in the Campylobacter-selective medium may be responsible for low recovery of the *A. sobria* strains in their study.

Discordant information exists in the literature on the activity of the aminoglycosides. The observations of Pasquale *et al.* (1994), von Graevenitz and Altwegg (1991) are in agreement with the results reported here. On the other hand, Rashad and Abdelkareem (1995) found some aeromonads to be resistant to amikacin. Most of the isolates of *A. hydrophila*, *A. caviae* and *A. veronii bv. sobria* turned out to be sensitive to several cephalosporins. The activity of some of the cephalosporins (cefazolin, cephalexins, cephalothin and cefoxitin) varied for the different isolates but was much greater against *A. veronii bv. sobria*. This confirms the proposal of Janda and Motyl (1985) that cephalothin susceptibility could be a useful phenotypic marker for identification of *A. sobria*.

The majority of the strains showed resistance to piperacillin, which inhibited growth of some to a variable extent. All isolates were also resistant to vancomycin and clindamycin with one exception. Krovacek *et al.* (1992) also found all strains of *A. hydrophila* and *A. sobria* to be resistant to penicillin and related antibiotics.

The present observations on the quinolone, ciprofloxacin are similar to those of Pasquale *et al*. (1994) and Hatha *et al.* (2005) who found all strains of *A. hydrophila* to be sensitive. According to von Graevenitz and Altwegg (1991), all aeromonads are sensitive to this drug.

There is some disagreement in the literature regarding the sensitivity of the aeromonads to sulfamethoxazole-trimethoprim complex. Several authors have reported that all strains *of A. hydrophila*, *A. sobria* and *A. caviae* are resistant to it. In the present work, sulfamethoxazole alone showed poor activity but as a complex SXT, its effectiveness against the isolates improved significantly (see also von Graevenitz and Altwegg, 1991).

Tetracycline, chloramphenicol and nitrofurantoin have been reported to give excellent activity against the aeromonads (von Graevenitz *et al.*, 1991; Pasquale *et al.,* 1994; Vivekanandhan *et al.,* 2002) as shown in the present study as well. Carnahan *et al.,* (1991c) have shown that 13 of their strains of *A. trota* are sensitive to cefuroxime, ceftrioxone, ciprofloxacin, ticarcillin, mezlocillin, amikacin, gentamicin, SXT, piperacillin and tobramycin. These results are nearly comparable to those reported here. While all strains tuned out to be sensitive to tetracycline and cefoxitin in this study, Carnahan and coworkers (1991c) noted that some of their isolates resisted these drugs. These workers also noted a higher percentage of susceptibility of *A. trota* to some of these drugs than reported in the present work. *A. schubertii* also showed sensitivity to the different antibiotics. In fact, both strains tested here were sensitive to most of the drugs but showed resistance to ticarcillin and piperacillin which contrasts with the results of Carnahan *et al.* (1991c) who noted that all of their strains are sensitive to these drugs. The results of Carnahan *et al.* (1991c) regarding *A. jandaei* also support the present observations for this species with only minor differences.

In conclusion, based on present investigations it is suggested that many antibiotics such as cefuroxime, ceftrioxone, colistin, amikacin, gentamicin, tetracycline, chloramphenicol, nitrofurantoin, cefotaxime and tobramycin could be effectively used to control the aeromonads as >75% of the test strains have been found sensitive to these antibiotics *in vitro*.

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