African Journal of Microbiology Research Vol. 3(12) pp. 903-913, December, 2009
Available online http://www.academicjournals.org/ajmr
ISSN 1996-0808 ©2009 Academic Journals

Full Length Research Paper

Antimicrobial susceptibility testing of *Aeromonas hydrophila* isolated from Limpopo Province, South Africa using VITEK 2 system, Micro Scan WalkAway, disk diffusion and E-test method

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Accepted 2 September, 2009

A total of 300 isolates of *Aeromonas Hydrophila* isolated from water and stool samples were tested using the Vitek 2 system, disk diffusion, MicroScan Walkaway and E-test for antimicrobial susceptibility testing. For the total of 34 antimicrobial tested, the MICs agreement was 99.7% for isolates from all sources. Almost 100% of isolates were resistant to ampicillin using both methods with the MIC ranging from 1 to 64 µg/ml. Overall; the agreement of the AST results among all four methods for the drugs tested was (100%) Aminoglycosides, (100%) Carbapenems, (100%) Monobactams, 93% Cephalosporins and 89.4% Beta-lactam/ Beta-lactam inhibitors. Overall agreement between the disk diffusion, MicroScan Walkaway and Vitek methods was 98%, respectively. In general, discrepancies among the methods were due to isolates being interpreted as intermediately susceptible or due to an increased number of resistances detected with disk diffusion and a lower number with Vitek and MicroScan Walkaway.

Key words: Vitek 2 system, E-Test, MICs, micro scan walkway, diarrhea.

INTRODUCTION

Aeromonas species are microbial etiological agents of diarrhoea particularly in developing countries, where diarrhoeal diseases constitute a very important cause of morbidity and mortality among children and young adults (WHO, 2002). It has been reported that more than 800 millions cases of diarrhoea occur annually in developing countries particularly in rural areas; accounting for about 4.5 million deaths (Oyofo et al., 2002). Children below the age of five especially those in areas devoid of access to potable water supply and sanitation, immune incompetent patients and elderly people are extremely prone to the devastating effects of diarrhoea which might be transmitted by contaminated food and water (Obi et al., 2003). Classical microbial agents of diarrhoea include viruses namely rotaviruses, Norwalk viruses, adenoviruses, calici like viruses; parasites such as *Giardia lamblia*, *Cryptosporidium parvum*, *Entamoeba histolytica* and bacteria such as *Escherichia coli*, S*almonella*, *Shigella*, V*ibrio cholerae*, *Aeromonas, klebsiella* and *Campylobacter* species (Obi et al., 2003, Samie et al., 2007). Although viruses, particularly rotaviruses, are frequently incriminated in childhood diarrhoea, bacteria and parasitic agents such as *Campylobacter* and *E. histolytica*, constitute major causes of diarrhea in developing countries (Samie et al., 2006).

However, incriminating evidence suggest that some emerging agents of diarrhea, such as *Aeromonas* species accounts for a substantial degree of morbidity and mortality in different age groups. Thus, diarrhoeal agent of concern in this study is *A. hydrophila*. *Aeromonas* species are important opportunistic pathogens in HIV/AIDS disease and may cause a septicaemic illnessin the absence of enteric disease (Manfredi et al., 2002). *Aeromonas* species have emerged as significant causes of gastroenteritis and when clinical laboratories include screenings for *Aeromonas* in routine enteric

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culture procedures, the percentage-recovery for this organism often exceeds that of *Salmonella* and *Shigella* combined (Wasf et al., 2000). The isolation rate of *Aeromonas* in many developing countries may range from 5 to 28% in clinical isolates (Oberhelman and Taylor, 2000). In food samples particularly poultry, *Aeromonas* has been isola-ted in rates as high as 82% in broilers in Senegal (Cardinale et al., 2003) and in 77% of chicken samples in Kenya (Osano and Arimi, 1999). In the Venda region of South Africa, *Aeromonas* species were isolated from clinical and environmental samples (Obi et al., 2007).

However studies on *Aeromonas* species have received little attention in South Africa. The management of diarrhoea may depend on the use of antibiotics for bacterial agents such as *Aeromonas* species. Macrolides, cephalosporins and fluoroquinolones are commonly used drugs in the treatment of severe *Aeromonas* infections. However, resistance to these antimicrobial agents have been described worldwide (Engberg et al., 2001; Cardinale et al., 2003; Upcraft and Upcraft, 2001) and has increased tremendously. Resistance to another macrolide, azithromycin was found in 7 to 15% of *Aeromonas* isolates in 1994 and 1995 in Thailand (Hoge et al., 1998). Cardinale et al. *(*2003) reported an increase in resistance to fluoroquinolone in Senegal. Multidrug resistance has also been described for *Aeromonas* due to over expression of the EHPgp 1 and 5 genes as well as the production of superoxide dismutase (Higgins, 1993). The increasing resistance of microorganisms to antimicrobial agents has necessitated the search for novel and more effective antimicrobial compounds (Obi et al., 2003). Hence the aim of this study was to determine antimicrobial susceptibility testing of *Aeromo-nas hydrophila*, isolated from Limpopo Province, South Africa by the VITEK 2 system and E-test methods.

MATERIALS AND METHODS

A total of 1,369 samples (660 stool samples and 709 water samples) were collected during 2005 and 2006 in Limpopo Province and were screened for the presence of *Aeromonas* species. Stool specimen with and without diarrhea were cultured on blood agar (Oxoid Ltd, Basingstoke, UK) and MacConkey agar (Difco/BD Diagnostics Systems, Sparks, MI, USA) and water samples were plated on Cysteine Lactose Electrolytes Deficient (CLED) agar and MacConkey agar (Difco/BD Diagnostics Systems, Sparks, MI, USA). A total of 300 isolates were used in this study of which 150 were isolated from stool samples and 150 were isolated from water samples respectively.

Isolated strains were stored in tubes containing 1.5 ml Brain Heart Infusion broth with 10% v/v glycerol at -70°C for further analysis. The isolates were identified using biochemical tests and confirmed using the API 20E and API 20 NE identification systems (bioMerieux, Marcy-l'Etoile, France). The isolates were further identified by the VITEK 2 system.

Antibiotic susceptibility testing

Microdilution and disk diffusion were performed as described by the National Committee for Clinical Laboratory Standards. The susceptibility of the *A. hydrophila* to antimicrobial agents was examined by an agar diffusion method using paper disks containing the following of antibiotics concentration: amikacin (30 µg), ampicillin (10 µg), gentamicin(10 µg), cefalotin(30 µg), cefotaxime (30 µg), cefoxitin (30 µg), ceftazidime(30 µg) ,piperacillin/tazobactam(100/10µg), amoxicilin/clavulanicacid (20/10µg), ofloxacin(5 µg), imipenem (10µg), cefuroxime (30 µg), cefepime (30 µg), meropeenem (10 µg), cefpdoxime (10 µg), trimethoprim/sulfathoxazole (1.25/23.75 µg), nitrofurantoin (300 µg), norfloxacin (10 µg), ofloxacin (5 µg), piperacillin (100 µg), tobramycin (10 µg), colistin (10 µg), aztreonam (30 µg), cefpirome (30 µg), isepamicin (30 µg), netilmicin (30 µg), pefloxacin (30 µg) , ticarcillin (75 µg), ticarcillin/clavulanic acid (75/10 µg), cefaclor (30 µg) , nalidixic acid (30 µg) and ertapenem (10 µg). These antimicrobial agents were selected on the basis of antimicrobial agents which can be measured by the VITEK 2 system card according to NCCLS guideline M7-A5 (NCCLS, 2003).

VITEK 2 system susceptibility tests

Antimicrobial susceptibilities of the test organisms were determined using the VITEK 2 system software version 1.02 (bioMérieux) according to the manufacturer's recommendations. The test organisms from colonies grown on 5% horse blood agar after 18 h incubation were suspended in sterilized physiological saline to 0.5 McFarland standards. Approximately 2 ml of this suspension was automatically loaded into the VITEK 2 ID GNB (identification-Gram-negative bacilli) and AST (antimicrobial susceptibility testing)-GN04 cards (for Gram-negative bacilli).

Micro scan walkway susceptibility tests

MicroScan (Dade Behring, Inc., W. Sacramento, Calif.) susceptibility tests were performed according to the manufacturers' directions. The identity of the bacteria was determined using the MicroScan WalkAway–96 system with conventional gram-negative breakpoint panels (NBPC 11). Briefly, bacterial suspension was prepared by inoculating 3 ml sterile water with colony isolates and adjusting the suspension to a 0.5 McFarland Standard. The prepared plates were then incubated at 37°C for 24 h, and zones of inhibition were calculated by measuring the diameter (mm) of the inhibited growth zone.

E-Test susceptibility tests

E test was performed according to the manufacturer's instruction. Briefly, an overnight culture of the bacteria diluted to a 0.5 McFarland turbidity standard was used to inoculate Mueller-Hinton agar plate (Oxoid, Basingstoke, UK). After drying, the E-test strips were applied on the plates and incubated overnight at 37°C. The minimum inhibitory concentrations (MICs) on both ends were read on the intersection of the inhibition ellipse and the E test-strip edge. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as positive controls. These tests were performed according to NCCLS M7-A5 guidelines (NCCLS, 2003) and M100-S10 guidelines (NCCLS, 2003), respectively. The MICs were interpreted using the recommended NCCLS thresholds.

Data analysis

All data analysis was performed by using the SAS System for Windows, release 6.12 (SAS Institute, Cary, N.C.). The resistance breakpoints used in this study was those according to National Committee for Clinical Laboratory Standards (NCCLS, 2003).

These breakpoints were used to calculate; very major, major, and minor errors between the E-Test, MicroScan, and Vitek results. Very major errors occurred with organisms for which MICs indicated resistance by Vitek and susceptibility by the Microscan and E-Test method. Major errors occurred with organisms for which MICs indicated susceptibility by Microscan and resistance by the E-Test and Vitek method. Minor errors occurred with organisms for which MICs indicated intermediate resistance by one or two methods and susceptibility or resistance by the other method. Denominators for calculating error rates, were as follows: the number of resistant isolates (very major error rate), the number of susceptible isolates (major error rate), and the total number of isolates tested (minor error rate).

Statistical analysis

Simple linear regression analysis was applied to define linear functions correlating the zone of inhibition (mm) with MICs obtained by E-test (mg/l). The E-test and agar dilution variables were linearized by logarithmic conversions.

The E-test results were also compared to the zones of inhibition using the method of least squares as applied to computers. The strength of the linear association between pairs of variables was determined by coefficients of determination (R-square): R-square ≥50%, strong correlation; R-square ≥25 – <50%, moderate correlation; and R-square < 25%, weak correlation.

The validation of these linear models was carried out by F-test. All P values reported were two-tailed and values lower than 0.05 were considered significant. The data were analyzed with the Minitab statistical package.

RESULTS

Three hundred isolates of *A. hydrophila* which 150 were isolated from stool samples and the other 150 isolated from water samples were tested against various types of antimicrobials using different methodologies such as E-Test, disk diffusion, MicroScan conventional panels and Vitek cards (Tables 1 - 6).

In a comparison of the overall error rates among the different methods and antimicrobial agents for isolates from stool samples, there were a limited number of very major errors for most of the agents tested. The exceptions were with the Vitek, in which three (2%) very major errors for Cefuroxime and thirteen (8.7%) for Piperacillin/tazobactam were detected. Only one (n = 2; 1.3%) major errors was detected for Imipenem in the Vitek; however, there were major errors in the results obtained by MicroScan Walkaway and E-Test. The major error rate for MicroScan Walkaway was highest for Piperacillin/tazobactam (n = 5; 3.3%) and Norfloxacin (n = 1; 90.7%) and in the E-Test, the major error rate was the highest for Tobramycin (n =3; 2%). The highest minor error rate was detected in the Vitek with Aztreonam (n = 38; 25.3%), in MicroScan Walkaway with Norfloxacin (n = 12; 8%) and in E-Test with Aztreonam (n = 43; 28.7%). Overall, there was more than 98.0% agreement with E-Test, MicroScan walkaway and the Vitek methods, respectively.

However, in comparison, the isolates from water samples, there were no very major and major errors for all the agents tested. The highest minor error rate was detected in the Vitek with Trimethoprim/sulfathoxazole (n = 3; 2%), in MicroScan Walkaway with Norfloxacin (n = 3; 2%) and in E-Test with Cefepime (n = 2; 1.3%). Overall, there was more than 99.7% agreement with E-Test, MicroScan Walkaway and the Vitek methods, respectively.

In a comparison of the overall antimicrobial and interpretation among the different methods and antimicrobial agents for isolates from stool samples, there were a limited number of resistances to most of the agents tested. The exceptions were with the Disk diffusion, in which most Quinolones were resistance which range from 4 to 15%. Only two of five test aminoglycosides showed some resistance with amikacin, 7% and Gentamicin, 5%. Only 62% of ampicillin showed resistance amongst all Beta-lactam penicillins tested. No resistance was detected on Beta-lactam/ Beta-lactam inhibitors, Carbapenems, Monobactams, Folate antagonists and other such as colistin tested. However, Cephalosporins showed some resisitance which ranges from 1 to 18%. The Vitek, MicroScan Walkaway and E-Test also show some resistance Quinolones with the E-Test showing less resistance. The aminoglycosides also showed some resistance for both three methods used ranging from 1 to 23%, respectively. Amongst all tested Beta-lactam penicillins by Vitek, MicroScan Walkaway and E-Test ampicillin showed resistance, 97 to 100%, respectively. No resistance was detected on Beta-lactam/ Beta-lactam inhibitors, Carbapenems, Monobactams, Folate antagonists and other such as colistin and Nitrofurantoin tested. However, Cephalosporins showed some resisitance which ranges from 1 to 16%. Overall, there was more than 98.0% agreement with E-Test, MicroScan Walkaway and the Vitek methods, respectively with about 2.0% disk diffusion disagreement.

In a comparison of the overall percentage resistance among the different methods and antimicrobial agents for isolates from water samples, there were resistance antimicrobial agents tested, with the exception of ampicilin which show resistance of 94 to 100%. Overall, there was more than 99.9% agreement with Disk diffusion, E-Test, MicroScan Walkaway and the Vitek methods, respectively.

The MICs was done using microdilution method, for all tested (stool isolates) antimicrobials. The MICs value for Quinolones range between 1 - 64 µg/ml with the exception of Nalidixic Acid which range from 1 - 128 µg/ml. The MICs value of all tested Aminoglycosides range from ≤1 to 64 µg/ml with the exception of Tobramycin which was tested from 1 to 64 µg/ml. The MICs value of ampicilin was the highest which range from 128 to ≥512 µg/ml. The MICs value of Beta lactam/Beta-lactam inhibitors range from ≤1 to 64 µg/ml and Carbapenems's MICs value ranges from 1 to 64 µg/ml. Cephalosporins MICs ranges from 1 to 64 µg/ml with the exception of

Table 1. Comparison of different methods on 150 of *Aeromonas hydrophila* isolated from stool samples.

Antimicrobial agent and error type	VITEK 2 system			Micro Scan Walkway			E Test		
	Very major	Major	Minor	Very major	Major	Minor	Very major	Major	Minor
Quinolones									
Ciprofloxacin	0	0	2	0	0	0	0	0	0
Norfloxacin	0	0	0	0	1	12	0	0	23
Ofloxacin	0	0	0	0	0	0	0	0	0
Pefloxacin	0	0	0	n/a	n/a	n/a	0	0	14
Nalidixic Acid	0	0	0	n/a	n/a	n/a	0	0	0
Aminoglycosides									
Amikacin	0	0	3	0	0	0	0	0	1
Gentamicin	0	0	0	0	0	0	0	0	0
Netilmicin	0	0	0	n/a	n/a	n/a	0	0	0
Isepamicin	0	0	0	n/a	n/a	n/a	0	0	0
Tobramycin	0	0	0	0	0	0	0	3	0
Beta-lactam penicillins									
Ampicillin	0	0	21	0	0	7	0	0	0
Piperacillin	0	0	0	n/a	n/a	n/a	0	0	0
Ticarcillin	0	0	0	n/a	n/a	n/a	0	0	3
Beta-lactam/Beta-lactam inhibitors									
Piperacillin/tazobactam	13	0	3	0	5	0	0	0	0
Amoxicillin/clavulanic acid	0	0	0	0	0	0	0	0	17
Ticarcillin/calvunic acid	0	0	0	n/a	n/a	n/a	0	0	0
Carbapenems									
Imipenem	0	2	0	0	0	0	0	0	15
Meropenem	0	0	26	n/a	n/a	n/a	0	0	31
Ertapenem	0	0	0	0	0	0	0	0	0
Cephalosporins									
Cefalotin	0	0	10	0	0	1	0	0	0
Cefotaxime	0	0	0	0	0	0	0	0	23
Cefoxitin	0	0	0	0	0	0	0	0	12
Ceftazidime	0	0	13	0	0	0	0	0	0
Cefuroxime	3	0	0	0	0	0	0	0	0
Cefepime	0	0	5	0	0	0	0	0	0
Cefpirome	0	0	0	n/a	n/a	n/a	0	0	0
Cefaclor	0	0	0	n/a	n/a	n/a	0	0	0
Cefpodoxime	0	0	0	n/a	n/a	n/a	0	0	0
Monobactams									
Aztreonam	0	0	38	0	0	0	0	0	43
Folate antagonists									
Trimethoprim/sulfathoxazole	0	0	0	0	0	0	0	0	0
Others									
Colistin	0	0	0	n/a	n/a	n/a	0	0	12
Nitrofurantoin	0	0	0	0	0	0	0	0	0

Table 2. Percentage susceptibility of 150 of *Aeromonas hydrophila* isolates from stool samples.

Antimicrobial and interpretation	Disk diffusion			VITEK 2 system			MicroScan WalkAway			E Test		
	S	I	R	S	I	R	S	I	R	S	I	R
Quinolones												
Ciprofloxacin	73	17	10	88	2	10	78	12	10	100	0	0
Norfloxacin	67	23	10	93	1	6	77	16	7	100	0	0
Ofloxacin	80	5	15	84	1	15	89	2	9	98	1	1
Pefloxacin	96	0	4	100	0	0	n/a	n/a	n/a	100	0	0
Nalidixic Acid	87	3	10	87	5	8	n/a	n/a	n/a	97	2	1
Aminoglycosides												
Amikacin	67	26	7	71	23	6	84	2	14	76	1	23
Gentamicin	78	17	5	83	14	3	76	3	20	77	4	19
Netilmicin	100	0	0	100	0	0	n/a	n/a	n/a	100	0	0
Isepamicin	100	0	0	100	0	0	n/a	n/a	n/a	100	0	0
Tobramycin	89	3	0	92	5	3	88	3	9	91	3	6
Beta-lactam penicillins												
Ampicillin	25	13	62	0	3	97	0	0	100	0	0	100
Piperacillin	98	2	0	93	2	5	n/a	n/a	n/a	100	0	0
Ticarcillin	99	1	0	100	0	0	n/a	n/a	n/a	100	0	0
Beta-lactam/ Beta-lactam inhibitors												
Piperacillin/tazobactam	100	0	0	100	0	0	100	0	0	100	0	0
Amoxicillin/clavulanic acid	100	0	0	100	0	0	100	0	0	100	0	0
Ticarcillin/calvunic acid	100	0	0	100	0	0	n/a	n/a	n/a	100	0	0
Carbapenems												
Imipenem	100	0	0	98	2	0	100	0	0	100	0	0
Meropenem	100	0	0	100	0	0	n/a	n/a	n/a	100	0	0
Ertapenem	100	0	0	100	0	0	99	1	0	100	0	0
Cephalosporins												
Cefalotin	78	12	10	88	9	3	82	5	13	86	2	12
Cefotaxime	71	10	19	86	6	8	77	8	15	81	7	12
Cefoxitin	90	6	4	100	0	0	n/a	n/a	n/a	98	1	1
Ceftazidime	98	1	1	100	0	0	100	0	0	100	0	0
Cefuroxime	79	13	8	92	3	5	88	9	2	73	11	16
Cefepime	100	0	0	100	0	0	100	0	0	97	2	1
Cefpirome	100	0	0	100	0	0	n/a	n/a	n/a	100	0	0
Cefaclor	100	0	0	100	0	0	n/a	n/a	n/a	100	0	0
Cefpodoxime	73	9	18	70	16	14	n/a	n/a	n/a	89	5	6
Monobactams												
Aztreonam	100	0	0	98	2	0	100	0	0	100	0	0
Folate antagonists												
Trimethoprim/sulfathoxazole	100	0	0	100	0	0	100	0	0	100	0	0
Others												
Colistin	100	0	0	100	0	0	n/a	n/a	n/a	100	0	0
Nitrofurantoin	88	12	0	94	6	0	93	4	3	98	0	2

Table 3. MICs of 150 Aeromonas *hydrophila* isolated from stool samples.

Antimicrobial agent	No. of isolates at the following MIC(µg/ml)									
	≤1	2	4	8	16	32	64	128	256	≥512
Quinolones										
Ciprofloxacin	ND	112	31	4	1	1	1	0	0	0
Norfloxacin	ND	98	42	6	3	1	0	0	0	0
Ofloxacin	ND	108	33	3	6	0	0	0	0	0
Nalidixic Acid	ND	89	7	4	10	13	5	22	0	0
Aminoglycosides										
Amikacin	75	46	8	22	15	14	9	0	0	0
Gentamicin	16	31	52	14	9	11	17	0	0	0
Tobramycin	ND	96	17	4	12	21	0	0	0	0
Beta-lactam penicillins										
Ampicillin	0	0	0	0	0	0	0	48	13	89
Beta-lactam/ Beta-lactam inhibitors										
Piperacillin/tazobactam	ND	49	54	3	19	25	0	0	0	ND
Amoxicillin/clavulanic acid	ND	56	51	9	22	7	5	0	0	ND
Carbapenems										
Imipenem	56	38	19	17	7	9	4	0	0	0
Ertapenem	43	49	21	4	2	9	14	8	0	0
Cephalosporins										
Cefalotin	ND	31	100	8	5	4	2	0	0	0
Cefotaxime	ND	101	40	6	5	1	0	0	0	0
Cefoxitin	ND	93	38	9	10	0	0	0	0	0
Ceftazidime	ND	89	7	4	10	13	5	22	0	0
Cefuroxime	17	11	49	17	9	31	16	0	0	0
Cefepime	ND	89	23	21	11	5	0	0	0	0
Monobactams										
Aztreonam	ND	78	26	2	19	8	17	0	0	ND
Folate antagonists										
Trimethoprim/sulfathoxazole	ND	66	74	3	3	1	0	0	0	ND
Others										
Nitrofurantoin	44	24	40	7	11	22	0	0	0	0

ND =Not Done.

Ceftazidime which ranges from 1 to 128 µg/ml. Others tested ranges from 1 to 64 µg/ml. The MICs range for water isolates were 98 to 100% similar to that of stool isolates.

DISCUSSION

This study compared the results of four different antimicrobial susceptibility tests (AST) methods using three hundred isolates of *A. hydrophila* [(one hundred and fifty (150) were isolated from water sample and another one hundred and fifty (150) were isolate from stool sample]. The most significant discrepancies among the methods generally fell into two categories; the first was the detection of an errors followed by the MICs ranges. Overall, the number of “Very major” was with the Vitek, in which three (2%) “Very major errors” for Cefuroxime and thirteen (8.7%) for Piperacillin/tazobactam were detected. Only one (n = 2; 1.3%) “Major errors” was detected for Imipenem in the Vitek; however, there were major errors in the results obtained by Micro scan Walkway and E-Test. The major error rate for Micro Scan Walkway was highest for Piperacillin/tazobactam (n = 5; 3.3%) and Norfloxacin (n = 1; 90.7%), and in the E-Test, the major error rate was the highest for Tobramycin (n = 3; 2%). There were also a number of “Minor errors” detected in the study that were more widely distributed among the various typing methods. Of the minor errors, highest minor error rate was detected in the Vitek with Aztreonam (n = 38; 25.3 %). These discrepancies, in part, may be due to the interpretation of

Table 4. Comparison of different methods against 150 *aeromonas hydrophila* isolated from water samples.

Antimicrobial agent and error type	VITEK 2 system			MicroScan WalkAway			E Test		
	Very major	Major	Minor	Very major	Major	Minor	Very major	Major	Minor
Quinolones									
Ciprofloxacin	0	0	0	0	0	0	0	0	0
Norfloxacin	0	0	0	0	0	0	0	0	0
Ofloxacin	0	0	0	0	0	0	0	0	0
Pefloxacin	0	0	0	n/a	n/a	n/a	0	0	0
Nalidixic Acid	0	0	0	n/a	n/a	n/a	0	0	0
Aminoglycosides									
Amikacin	0	0	0	0	0	0	0	0	0
Gentamicin	0	0	0	0	0	0	0	0	0
Netilmicin	0	0	0	n/a	n/a	n/a	0	0	0
Isepamicin	0	0	0	n/a	n/a	n/a	0	0	0
Tobramycin	0	0	0	0	0	0	0	0	0
Beta-lactam penicillins									
Ampicillin	0	0	0	0	0	0	0	0	0
Piperacillin	0	0	0	n/a	n/a	n/a	0	0	0
Ticarcillin	0	0	0	n/a	n/a	n/a	0	0	0
Beta-lactam/ Beta-lactam inhibitors									
Piperacillin/tazobactam	0	0	0	0	0	0	0	0	0
Amoxicillin/clavulanic acid	0	0	0	0	0	0	0	0	0
Ticarcillin/calvunic acid	0	0	0	n/a	n/a	n/a	0	0	0
Carbapenems									
Imipenem	0	0	**0**	0	0	0	0	0	0
Meropenem	0	0	0	n/a	n/a	n/a	0	0	0
Ertapenem	0	0	0	0	0	0	0	0	0
Cephalosporins									
Cefalotin	0	0	0	0	0	0	0	0	0
Cefotaxime	0	0	0	0	0	0	0	0	0
Cefoxitin	0	0	0	0	0	0	0	0	1
Ceftazidime	0	0	0	0	0	0	0	0	0
Cefuroxime	0	0	0	0	0	0	0	0	0
Cefepime	0	0	0	0	0	0	0	0	2
Cefpirome	0	0	0	n/a	n/a	n/a	0	0	0
Cefaclor	0	0	0	n/a	n/a	n/a	0	0	0
Cefpodoxime	0	0	0	n/a	n/a	n/a	0	0	0
Monobactams									
Aztreonam	0	0	0	0	0	3	0	0	0
Folate antagonists									
Trimethoprim/sulfathoxazole	0	0	3	0	0	0	0	0	0
Others									
Colistin	0	0	0	n/a	n/a	n/a	0	0	0
Nitrofurantoin	0	0	0	0	0	2	0	0	0

Table 5. Percentage susceptibility of 150 *Aeromonas hydrophila* isolated from water samples.

Antimicrobial and interpretation	Disk diffusion			VITEK 2 system			MicroScan WalkAway			E Test		
	S	I	R	S	I	R	S	I	R	S	I	R
Quinolones												
Ciprofloxacin	25	75	0	97	3	0	100	0	0	100	0	0
Norfloxacin	98	2	0	93	7	0	n/a	n/a	n/a	100	0	0
Ofloxacin	99	1	0	100	0	0	n/a	n/a	n/a	100	0	0
Pefloxacin	87	13	0	87	12	0	n/a	n/a	n/a	97	3	0
Nalidixic Acid	81	20	5	83	17	0	76	23	0	77	23	0
Aminoglycosides												
Amikacin	100	0	0	100	0	0	100	0	0	100	0	0
Gentamicin	100	0	0	100	0	0	100	0	0	100	0	0
Netilmicin	100	0	0	100	0	0	n/a	n/a	n/a	100	0	0
Isepamicin	100	0	0	100	0	0	n/a	n/a	n/a	100	0	0
Tobramycin	89	3	0	92	8	0	88	12	0	91	9	0
Beta-lactam penicillins												
Ampicillin	0	3	97	0	0	100	0	0	100	0	0	100
Piperacillin	100	0	0	100	0	0	100	0	0	100	0	0
Ticarcillin	100	0	0	100	0	0	n/a	n/a	n/a	100	0	0
Beta-lactam/ Beta-lactam inhibitors												
Piperacillin/tazobactam	100	0	0	100	0	**0**	100	0	0	100	0	0
Amoxicillin/clavulanic acid	100	0	0	100	0	0	98	2	0	100	0	0
Ticarcillin/calvunic acid	100	0	0	100	0	0	n/a	n/a	n/a	100	0	0
Carbapenems												
Imipenem	100	0	0	100	0	0	100	0	0	97	3	0
Meropenem	100	0	0	100	0	0	n/a	n/a	n/a	100	0	0
Ertapenem	100	0	0	100	0	0	100	0	n/a	100	0	0
Cephalosporins												
Cefalotin	100	0	0	100	0	0	100	0	0	100	0	0
Cefotaxime	100	0	0	100	0	0	100	0	0	100	0	0
Cefoxitin	100	0	0	100	0	0	n/a	n/a	n/a	100	0	0
Ceftazidime	100	0	0	100	0	0	100	0	0	100	0	0
Cefuroxime	100	0	0	100	0	0	99	0	1	100	0	0
Cefepime	100	0	0	100	0	0	100	0	0	100	0	0
Cefpirome	100	0	0	100	0	0	n/a	n/a	n/a	100	0	0
Cefaclor	100	0	0	100	0	0	n/a	n/a	n/a	100	0	0
Cefpodoxime	100	0	0	100	0	0	n/a	n/a	n/a	100	0	0
Monobactams												
Aztreonam	100	0	0	100	0	0	100	0	0	100	0	0
Folate antagonists												
Trimethoprim/sulfathoxazole	100	0	0	100	0	0	100	0	0	100	0	0
Others												
Colistin	100	0	0	100	0	0	n/a	n/a	n/a	100	0	0
Nitrofurantoin	94	6	0	100	0	0	96	4	0	100	0	0

Table 6. MIC (µg/ml) of 150 aeromonas hydrophila isolated from water samples.

Antimicrobial agent	No. of isolates at the following MIC(µg/ml)									
	≤1	2	4	8	16	32	64	128	256	≥512
Quinolones										
Ciprofloxacin	ND	126	6	3	5	7	4	0	0	0
Norfloxacin	ND	106	23	6	4	8	3	0	0	0
Ofloxacin	ND	108	33	3	6	0	0	0	0	0
Nalidixic Acid	ND	49	72	5	23	1	0	0	0	0
Aminoglycosides										
Amikacin	34	66	18	30	0	0	0	0	0	0
Gentamicin	66	45	4	7	9	11	8	0	0	0
Tobramycin	ND	18	95	7	11	19	0	0	0	0
Beta-lactam penicillins										
Ampicillin	0	0	0	0	0	0	0	0	37	113
Beta-lactam/ Beta-lactam inhibitors										
Piperacillin/tazobactam	ND	100	33	17	0	0	0	0	0	ND
Amoxicillin/clavulanic acid	ND	112	14	8	16	0	0	0	0	ND
Carbapenems										
Imipenem	132	15	3	0	0	0	0	0	0	0
Ertapenem	143	2	3	1	0	0	0	0	0	0
Cephalosporins										
Cefalotin	ND	101	29	6	10	4	0	0	0	0
Cefotaxime	ND	83	44	18	5	0	0	0	0	0
Cefoxitin	ND	100	33	4	13	0	0	0	0	0
Ceftazidime	ND	111	8	2	15	10	4	0	0	0
Cefuroxime	88	6	47	9	0	0	0	0	0	0
Cefepime	ND	134	16	0	0	0	0	0	0	0
Monobactams										
Aztreonam	ND	133	12	2	3	0	0	0	0	ND
Folate antagonists										
Trimethoprim/sulfathoxazole	ND	99	8	41	2	0	0	0	0	ND
Others										
Nitrofurantoin	90	23	20	10	6	0	0	0	0	0

the results, because in a number of cases the resistance detected was just over the MIC resistance breakpoint, and the susceptible isolates were detected below the intermediate-susceptible range with other methods. While there were some discrepancies, overall, there was a greater than 98% agreement between each testing method.

When the results of this study were compared to other AST comparison studies, the results were relatively similar. The error rates reported by Guthrie et al. (1999) and Rajesh et al. (2007) had a similar pattern to the present study. Our findings were also similar with the findings by Guthrie et al. (1999) for trimethoprim /sulfamethoxazole, Cefuroxime and Piperacillin/ tazobactam. Karlowsky et al. (2003), also examined susceptibility testing using different methodologies in gram negative organisms; their findings were also similar with our findings, with overall categorical error rates of around 2% for Vitek and broth micro dilution testing, which was similar to the 2.1 to 3.3% range in our study.

The findings of only a single “Very major” and a single “Major error” for trimethoprim/sulfamethoxazole was notable because trimethoprim/sulfamethoxazole is one of the agents of choice for the treatment of invasive salmonellosis (Rajesh et al., 2007). Results indicating that Cefuroxime and Piperacillin/tazobactam had the highest error rates which were also interesting because these drugs are used for the treatment of enterobacterciae. The second category was the demonstration of the minimum inhibitory concentrations which ranged between 1 to 64 µg/ml. Overall; our study confirmed that different methods were similar for susceptibility testing of *A. hydrophila* isolated from water and stool samples.

Conclusion

In spite of the overall agreement, our study indicates that Vitek 2 and Micro Scan Walkway could be used for identification and antimicrobial susceptibility of *A. hydrophila* isolates from both environmental and clinical sources. When the interpretative algorithms of this system for tests with *A. hydrophila* have been reassessed and the biases detected, corrected and the various types of errors detected minimized, alternative methods for routine AST of *A. hydrophila* isolates based on validated manual methods could only be limited to isolates from sterile sites such as blood culture, cerebrospinal fluid etc, thereby including automated systems for the routine AST of all *A. hydrophila* isolates. Overall, the study confirmed that the interpreted results of the methods were similar for susceptibility testing of *A. hydrophila* isolates with some noted exceptions. The interpreted results of the susceptibility testing methods evaluated in this study can be compared to results of other testing methods, thereby permitting greater sharing of susceptibility testing results among microbiologist.

ACKNOWLEDGMENTS

We thank Mr. Zebulon Kola for the assistance at NHLS laboratory where some work was undertaken. The National Research Foundation (NRF) for financial assistance.

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