African Journal of Biotechnology Vol. 8 (22), pp. 6388-6392, 16 November, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Plesiomonas shigelloides in stool samples of patients in the Venda Region: Possible considerations on pathogenicity and antibiogram profiles

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Accepted 18 April, 2008

This study determined the haemolytic, haemagglutinating and antibiotic susceptibility activities of *Plesiomonas shigelloides* isolated from stool samples of patients attending different health centers in the Venda region of South Africa. *P.shigelloides* was isolated and identified using the API 20E, API 20NE systems. Antibiotic susceptibility profiles of the isolates were determined using the disc diffusion method and analyzed according to NCCLS standards. The hemolytic and hemagglutination activities of the isolates on human, sheep, pig and chicken red blood cells were determined using the plate and slide methods. A total of 89 (13%) *P. shigelloide* were isolated from 660 samples. The hemolytic activities of the isolates were variable with no heamolysis on sheep red cells. 33 (37%) of isolates were beta lactamase producers. There was a high level of resistance to the penicilllins with 100% resistance to Penicillin G, Amoxicillin and Ampicillin. This study has demonstrated multiple resistance to different antibiotics and production of beta lactamase. Most of the isolates showed evidence of pathogenicity as demonstrated by hemolytic and haemagglutinating activities.

Key words: *Plesiomonas shigelloides*, stool samples, antibiograms, red blood cells, pathogenicity, haemolysis, haemagglutination.

INTRODUCTION

Plesiomonas shigelloides is gram-negative rod which is found in freshwater, freshwater fish, shellfish and different types of animals such as cattle, goats, swine, cats, dogs, monkeys, vultures, snakes, and toads. *P. shigelloides*, though aquatic in origin, is extensively distributed in the environment and is reportedly an agent of gastroenteritis (Salerno et al., 2007). Several reports have implicated the organism as a cause of diarrhoeal disease in humans and animals (Farmer et al., 1992). Human infections due to this bacterium are mostly waterborne. The organism may be present in unsanitary water, which has been used as drinking water, recreational water, or water used to rinse foods that are consumed without cooking or heating. *P. shigelloides* infections occur in summer months and correlate with environmental contamination of freshwater (rivers, streams and ponds). Outbreaks of gastroenteritis linked to consumption of oysters contaminated with *P. shigelloides* have given impetus to the public health significance of the organism (Miller and Koburger, 1985). *P. shigelloides* gastroenteritis is usually a mild self-limiting disease with fever, chills, abdominal pain, nausea, diarrhea, or vomiting; symptoms may begin 20 - 24 h after eating contaminated food or drinking contaminated water (Ueda et al., 1999). Diarrhea is usually watery, non-mucoid, and non-bloody and in severe cases may be greenish-yellow, foamy, and blood tinged.

Extra- intestinal complications such as septicemia (Nolte et al., 1988) may occur in people who are immunocompromised or seriously ill with cancer, blood disorders, or hepatobiliary disease. Inspite of the clinical significance of *P. shigelloides*, little is known about the genetic landscape, updated antibiograms, structure of the population and extent of infections caused by the organism

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(Salerno et al., 2007). As a prelude to unraveling the genetic diversity, which will impact on epidemiological control, a study on the prevalence, virulence potential and antibiograms of the organism is critical in order to gauge the extent of the problem, ascertain pathogenic factors and antibiograms of isolates for clinico-epidemiological relevance and for empiric management of infections requiring antibiotics.

In this first communication, we report on possible considerations of pathogenicity and antibiogram profiles of isolates as a prelude to an account of the genetic landscape of *P. shigelloides* in the Venda region of South Africa.

MATERIALS AND METHODS

Study site and patients

The study was carried out in the Vhembe district, Limpopo Province of South Africa. Diarrheic stool samples were collected from patients with diarrhea attending different hospitals in the region including Donald Frazer (Vhufuli), Elim, Tshilidzini, Siloam and Makhado hospitals. Each patient was given a 50 ml sterile plastic container and advised on how to collect the stool samples which were then kept in a cooler box with ice and taken to the Microbiology laboratory, University of Venda for microbiological analysis. A total 660 stool samples were collected and demographic information such as age and sex of the patients were recorded.

Culture methods

Specimens were investigated for the presence of *P. shigelloides*. The specimens were cultured using the method previously described (Obi et al., 2007a, 2007b). Briefly, freshly collected stool specimens were plated onto MacConkey agar and xylose deoxycholate citrate agar (XDCA) and incubated at 37°C for 24 h, the suspected colonies for *P. shigeloides were* tested for cytochrome oxidase (Kovac's method) as previously reported (Wong et al., 2003). The oxidase positive strains were further evaluated by triple sugar iron agar slants and API-20E and API-20NE commercial strips (Analytab products, Inc., plainview, N.Y). In addition, the sugars listed in Table 1 were also used in conjunction with commercial kit to identify *P. shielloides*.

Determination of beta-hemolytic activity

Beta-hemolytic activity was determination, using sheep blood agar, human blood agar, pig blood agar and chicken blood agar. Single colonies of each isolates were streaked across the various blood agar plates using sterile inoculating wire loops. The inoculated blood agar plates were incubated at 37ºC for 18 to 24 h. After incubation the hemolytic activities were determined by observing hemolysis on the blood agar (Obi et al., 2007a; Samie et al., 2007).

Beta-lactamase-production

The isolates were tested for the production of beta-lactamase. Two to three well-isolated colonies were picked and applied onto filter paper with applicator stick. A small quantity of Nitrocefin was drawn with a syringe and needle and a tiny drop of the fluid was applied onto each specimen (Samie et al., 2007). A change of colour of the filler paper from white to yellow indicated a positive reaction.

Antibiotic susceptibility and screening for extended-spectrum *β*-lactamases production

Susceptibility tests were performed using the Kirby-Bauer disk diffusion method and following NCCLS guidelines. The antibiotic-containing disks were obtained from oxoid and consisted of the following: Penicillin (PG, 10 units), Ciprofloxacin (CIP, 5 µg), Vancomycin (VA, 30 µg), Erythromycin (E, 30 µg), Tetracycline (T, 30 µg), fusidic acid (FC, 30 µg), Cloxcillin (CL, 30 µg), Rifampicin (RF, 30 µg), Meropenem (MEM, 10 µg), Imepenem (IMI, 10 µg), chloramphenicol (C, 10 µg) amoxacillin/clavulanic acid (AMC, 30 µg), nitrofurantoin (NI, 200 µg), Gentamycin (GN, 10 µg), Amikacin (AK, 10 µg), Ampicillin (AMP, 10 µg), Cefoxitin (FOX, 30 µg), Nalidixic acid (NA, 30 µg), Piperacillin/Tazobactam (PTZ, 110 µg), Doxycycline (DOX, 30 µg), Novobiocin (NO, 30 µg), Cotrimoxazole (TS, 25 µg), Cefotaxime (CTX, 30 µg), Amoxycilin (AM, 30 µg), cephazolin (CZ ,30 µg),cefuroxime(CXM, 30 µg), Cefepime(CPM, 30 µg) , and Cefotrizone (CRO, 30 µg). The MICs of cefotaxime, cefotaxime plus clavulanic acid, ceftazidime, ceftazidime plus clavulanic acid, cefepime, cefoxitin, nalidixic acid, ciprofloxacin, trimethoprim-sulfamethoxazole, imipenem, and meropenem were determined by E-test. The strips were used according to the manufacturer's instructions (AB Biodisk, Solna, Sweden). Antibiograms were ascertained as already described (Samie et al., 2007; Obi et al., 2007). After incubation the organisms were classified as sensitive (S), and resistant (R) (NCCLS, 2003). A total of 28 antibiotics were tested.

Determination of minimum inhibitory concentrations by microdilution

The minimum inhibitory concentrations (MICs) patterns of the isolates to 11 antibiotics were determined in microplates (NCCLS, 2003). The antibiotics tested were AMX (amoxicillin) ≤8; AMC (amoxicillin/clavulanic acid) ≤4; CTX (cefotaxime) ≤8; CTC (cefotaxime/clavulanic acid) ≤4; CRX (ceftriaxone) ≤8; CRC (ceftriaxone/clavulanic acid) ≤4; CXM (cefuroxime) ≤8; CXC (cefuroxime/clavulanic acid) ≤4; CAZ (ceftazidime) ≤8; CAC (ceftazidime/clavulanic acid) ≤4; PIP (piperacillin) ≤16; PIT (piperacillin/tazobactam) ≤8; and FOX (cefoxitin) ≤8.

Reference strains

Escherichia coli ATCC 25922 and klebscella pneumoniae ATCC 700603 were used as the reference strains for antimicrobial susceptibility testing.

RESULTS AND DISCUSSION

Out of a total of 660 samples collected from 580 (88%) females and 80 (12%) males with ages ranging from 1 - 60 years, only 89 (13%) were positive for *P.shigelloides*. Of 89 isolates of *P. shigelloides* isolated, 82 (92%) showed beta-haemolytsic activities on human red blood cells with 56 (63%) on chicken red blood cells whereas none (O%) showed haemolysis on sheep red blood cells and pig red blood cells. Only 33(37%) were beta-lactamase producers. Tables 2 and 3 show the MICs of selected antibiotics. The MICs were measured in µg/ml as well as antibiotic resistance of P. *shigelloides* to different antibiotics.

P. shigelloides is a bacterium of clinical significance but

Table 1. Profiles used for identification of *Plesiomonas shielloides.*

	LDC	ODC	ADH	Gas from glucose	Acid from: Sucrose	Acid from: Inositol	0/129 susceptibilty	Growth in: TCBS	Growth in: 0% NACL
	+	+	+	-	-	+	+	-	+
Plesiomonas shielloides	Oxidase	ß-haemolysis from sheep blood		Motility	DNAse	Indole	Esculin	Voges-Proskauer	
	+	-		+	-	+	+	-	

Abbreviations and symbols: LDC, lysine decarboxylase; ODC, ornithine decarboxylase, ADH arginine dihydrolase, TCBS, thiosulfate-citrate-bile salts-sucrose; 0/129, 2.4-diamino-6.7-diisopropylpteridine.

Table 2. Minimum inhibitory concentrations (µg/ml) of selected antibiotics.

P. shigelloides	AMX	CTX	CTC	CRX	CXM	CXC	CAC	PIT	FOX
P01	16	64	64	32	32	32	64	32	16
P02	32	32	32	32	32	32	32	32	16
P03	32	32	32	64	32	32	32	32	2
P04	32	64	64	64	32	32	64	32	2
P05	32	64	64	64	32	32	64	32	2
P06	32	16	16	32	32	32	16	32	8
P07	32	16	16	32	32	32	16	32	8
P08	32	32	32	32	64	64	32	64	8
P09	64	32	32	64	64	64	32	64	8
P10	64	16	16	64	64	64	16	64	2
P11	64	16	16	64	64	64	16	64	8
P12	64	16	16	64	32	32	16	32	16
P13	32	32	32	32	32	32	32	32	16
P14	32	32	32	32	64	64	32	64	4
P15	64	32	32	64	64	64	32	64	64
P16	64	32	32	64	16	16	32	16	8
P17	16	32	32	16	16	16	32	16	2
P18	16	64	64	16	32	32	64	32	2
P19	32	64	64	32	32	32	64	32	64
P20	32	64	64	32	16	16	64	16	8
P21	16	32	32	16	16	16	32	16	8
P22	16	32	32	16	16	16	32	16	2
P23	16	2	2	16	32	32	2	32	2
P24	32	8	8	32	32	32	8	32	8
P25	32	8	8	32	32	32	8	32	8
P26	32	8	8	32	32	32	8	32	8
P27	32	8	8	32	32	32	8	32	8
P28	32	8	8	32	64	64	8	64	2
P29	64	8	8	64	64	64	8	64	8
P30	64	16	16	64	64	64	16	64	16
P31	64	16	16	64	64	64	16	64	16
P32	64	4	4	32	64	64	4	64	4
P33	64	64	64	32	64	64	64	64	64
P34	64	8	8	32	64	64	8	64	8
P35	64	2	2	32	64	64	2	64	2
P36	64	2	2	64	16	16	2	16	2
P37	16	64	64	64	16	16	64	16	64
P38	16	32	8	64	16	16	8	16	8
P39	16	32	8	64	16	16	8	16	8

Table 2. Contd.

P40	16	32	8	32	16	16	32	16	8
P41	16	64	8	32	16	16	32	16	8
P42	16	64	8	32	32	32	32	32	8
P43	32	32	8	64	32	32	64	32	8
P44	32	32	8	64	32	32	64	32	8
P45	32	32	8	32	16	16	32	16	8

CTX = cefotaxim , CXM = cefuroxime, CTC = cefotaxime/clavulanic acid, CRX = cefriazone, CXC = cefuroxime/clavulanic, CAC = cefazidime/clavulanic acid, PIT = piperacillin/tazobactam, FOX = cefoxitin.
MIC is in µg/ml.
P: *Plesiomonas shigelloides* isolates (P1 – P45).

Table 3. Antibiotic susceptibility of *Plesiomonas shigelloides* isolated from stool samples in the Venda region.

Antibiotic	Code	Conc (µg)	Total no. of isolates	No resistant (%)
Penicillin G	PG	10	89	89 (100%)
Ciprofloxacin	CIP	5	89	16 (17%)
Vancomycin	VA	30	89	89 (100%)
Erythromycin	E	15	89	63 (71%)
Tetracycline	T	30	89	42 (47%)
Fusidic acid	FC	10	89	89 (100%)
Cloxacillin	CX	5	89	89 (100%)
Rifampicin	RP	5	89	89 (100%)
Meropenem	MEM	10	89	18 (20%)
Chloramphenicol	C	30	89	65 (73%)
Augmentin	AUG	30	89	69 (76%)
Nitrofurantoin	NI	300	89	89 (100%)
Gentamicin	GM	10	89	33 (23%)
Amikacin	AK	30	89	20 (22%)
Imipenem	IMI	10	89	16 (17%)
Ampicillin	AP	10	89	89 (100%)
Ceftriaxone	CRO	30	89	35 (39%)
Nalidixic acid	NA	30	89	47 (53%)
Pipevacillin+ TazoBactam	PTZ	110	89	22 (24%)
Doxycycline	DXT	30	89	28 (31%)
Novobiocin	NO	5	89	89 (100%)
Cotrimoxazole	TS	20	89	33 (23%)
Cefotaxime	CTX	30	89	42 (47%)
Cefuroxime	CXM	30	89	89 (63%)
Cefepime	CPM	30	89	18 (20%)
Cephazoline	CZ	30	89	16 (17%)
Cefoxitin	FOX	30	89	20 (22%)
Amoxicillin	A	10	89	89 (100%)

limited information is available on its antimicrobial susceptibility profiles and pathogenic factors in South Africa. The organism is implicated as a cause of sporadic and epidemic diarrhea in immunocompetent hosts and severe extraintestinal disease in immunocompromised individuals. A clinical approach to the treatment of *P. shigelloides* infections is based on the use of empiric antibiotic therapy especially in developing or rural communities without adequate facilities for antimicrobial susceptibility testing.

Antimicrobial susceptibility testing was performed on 89 stool isolates of *P. shigelloides* isolated from patients with diarrhea in the Venda region of South Africa using a standard agar dilution technique. Results obtained showed that imipenem, meropenem, cephazoline, cefepime, ciprofloxacin, gentamicin and cefoxitin may be useful in

the treatment of *P. shigelloides* infections. A study by Obi et al. (2007) demonstrated that the carbapenems and cephalosporins had the best *in vitro* activity against *Aeromonas* species, an organism related to P. shigelloides.

Results of our study are also in harmony with those of other researchers in terms of susceptibility of the organism to ciprofloxacin, gentamicin and imipenem (Kain and Kelly, 1989; Stock and Wiedemann, 2001; Wong et al., 2003). However, the organism showed resistance to multiple antibiotics such as penicillin, vancomycin amoxicillin, tetracycline and erythromycin.

Results also showed that majority of the isolates (92%) were beta-hemolytic and 63% showed haemolysis on human red blood cells. 37% of the isolates were beta-lactamase producers. Haemolysis and beta-lactamase production are indices of pathogenicity and results on these pathogenic factors simulate previous findings on related organisms (Obi et al., 2007; Samie et al; 2007; Wong et al., 2003).

In conclusion, meropenem and imipenem had good activity against *P. shigelloides* isolated from stool samples and may be useful in cases of gastrointestinal infection caused by *P. shigelloides*. However, further studies are needed to unravel genetic profiles of resistance genes as well as genes coding for virulence in *P. shigelloides*.

ACKNOWLEDGEMENTS

This study was supported by the National Research Foundation. The authors are grateful to individuals who assisted with the collection of samples.

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