CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.3484557

Available online at: http://www.iajps.com *Research Article*

PREVALENCE AND ANTIBIOGRAM OF PLESIOMONAS SHIGELLOIDES ISOLATED FROM HUMANS AND SOME ENVIRONMENTAL SOURCES

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Article Received: August 2019 **Accepted:** September 2019 **Published:** October 2019

Abstract:

Plesiomonas shigelloides are gram negative bacteria, implicated for numerous gastrointestinal infections such as diarrhoea. They are ubiquitous and have been successfully isolated from human as well as environmental samples. The rise in antibiotic resistance coupled with the increasing role of P. shigelloides in human diseases have made a study of the antibiogram of P. shigelloides important. The present study sought to isolate P. shigelloides from human and environmental samples and perform antibiogram profiling of the isolates. Thirty nine (39) P. shigelloides isolates from human and environmental (water, soil and seafood) samples collected in Port Harcourt were studied using standard microbiological procedures. P. shigelloides was isolated in 5(1.67%) of 300 patients, 24(15.0%) of 160 well-water samples, 8(16.0%) of 50 soil samples and 2(4.0%) of 50 seafood samples with significance difference ($p < 0.05$) in the various sources. In human samples, the susceptibility pattern of P. shigelloides was in the order Nitrofurantoin, Nalidixic acid, Colistin sulphate and Tetracycline (100%) > Co-trimoxazole (80%)>Sulphonamide (60%)> Ampicillin (20%)> Streptomycin (0%). Seafood isolates had a susceptibility pattern in a decreasing order; Nalidixic acid, Nitrofurantoin and Tetracycline (100%) > Ampicillin, Co-trimoxazole and Streptomycin (50%) > Streptomycin (0.00%). Soil isolates had a susceptibility pattern in the order; Nalidixic acid and Nitrofurantoin (100%)>Co-trimoxazole and Tetracycline (75%)> Sulphonamide (50%)>Ampicillin and Streptomycin (0.00%). Well-water and soil samples demonstrated a decreasing trend of resistance in the order Nitrofurantoin and Nalidixic acid (100%) > Tetracycline (45.83%)>Colistin-sulphate (41.67%) > Co-trimoxazole (37.5%) > Sulphonamide (29.17%) > Ampicillin (4.17%)>Streptomycin (0.00%). The present study highlight Nalidixic acid and Nitrofurantoin as drugs of choice for treating Plesiomonas infections as they recorded 100 % sensitivity to all isolates studied. Outright resistance was reported against ampicillin in soil samples, with all other antimicrobials recording varying degrees of sensitivity. None of the isolates were susceptible to streptomycin. Overall, human isolates were more sensitive to studied antibiotics compared to the environmental samples. This could be due to various factors leading to antibiotic resistance in the environment. There is a great need for improved environmental practices, precisely proper waste management could reduce incidents of antibiotic resistance.

Keywords: Plesiomonas shigelloides; antibiotic resistance; antibiogram, human samples, environmental samples

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Please cite this article in press Akani et al., ***Prevalence and Antibiogram of Plesiomonas Shigelloides Isolated from Humans and Some Environmental Sources****., Indo Am. J. P. Sci, 2019; 06(10).*

INTRODUCTION:

Plesiomonas shigelloides is the only member of the species *Plesiomonas* (1, 2). This genus is currently in the family Enterobacteriaceae but was formally in the family Vibrionaceae (1, 3). They are Gram negative, rod-shaped, ubiquitous bacteria. The name *Plesiomonas* demonstrates their nearness to the aeromonas group and shigelloides, their shigella-like appearance (1). Previously named *Aeromonas shigelloides*, they were named afresh on the discovery of difference in the guanine and cytosine contents (1).With optimal growth temperature ranging between 35 ºC to 39 ºC, these organisms are mesophiles and could grow between 8 ºC and 45 ºC. Similarly, they grow between 4.5 and 9 pH and salinities 0 % to 4% (1). *P. shigelloides* is found mostly in freshwater as well as its fauna such as, fish, shell fish (1, 4). They have also been reported in soil and other animals including cats, cattle, dogs, humans, goats, monkeys, vultures, snakes and toads (4).

Evidence of *P. shigelloides* involvement in gastroenteritis dates back to before 1940 when Paracolon was used to describe a myriad of enteric organisms with *P. shigelloides*, blindly identified as C27 Paracolon (1). Risks of *P. shigelloides* infection could increase by consumption of seafood, international travels, immunosuppression, consumption of untreated water as well as poor hygiene conditions (1). Further, temperature has also been highlighted as a disposing factor to *P. shigella* infections. Specifically, it is more in tropical regions with moderate temperature regimes, such as Nigeria. Incidence of its infection has also been reported higher in the summer (5). Mostly, infection of *P. shigelloides* is following consumption of contaminated, ill-treated or untreated water (5).

P. shigelloides has been isolated from a wide variety of human clinical samples including both intestinal (faeces or rectal swabs) and extra-intestinal samples. It has been isolated from faeces of human, presenting with or without diarrhoea, and/or vomiting (gastroenteritis) (6). Many reports have associated *P. shigelloides* with diarrhoea/gastroenteritis (4). However, isolation from the faeces of a diarrhoea case is not conclusive that the infection was caused solely by *P. shigelloides*; a temporal association does not prove causation. This is explained by the fact that *P. shigelloides* infections are mostly coinfections with other enteric pathogens (1). Further, some strains of *P.shigelloides* may cause diarrhoea in some people under certain conditions, but may not cause in others (7). *P. shigelloides* has been isolated from a wide variety of human extra-intestinal clinical specimen (8), often from those with an immune deficiency (9). *Plesiomonas* strains share antigens with *Shigella sonnei* and cross reactions with *Shigella* antisera may occur (10). *Plesiomonas* can be distinguished from *Shigella* in diarrheal stools by an oxidase test (11).

Primarily implicated in cases of diarrhoea (7, 8) *P. shigelloides* has also been reported in other diseases including hepatobiliary disease, bacteraemia and peritonitis (1). The epidemiological consequence of *P.shigelloides* cannot be over mentioned. This is evidenced by an even spread of this organism globally, highlighted by diseases caused. Janda *et al*. (1) posit that the organism has been reported in virtually all climes, across all age categories. Cases of *P. shigelloides* infection appears worse in warmer seasons as the freshwater temperatures increase and thus proliferation of the mesophiles.

Antibiotic resistance has become a great threat to public health globally (12, 13). This has resulted from many factors ranging from over prescription of drugs to use of the wrong drugs with no prior sensitivity/susceptibility testing. The place of antibiogram profile is integral in successful antibiotic therapy. Although *P. shigelloides* has shown to be highly susceptible, cases of antibiotic resistance have been reported (14, 15). The present study was aimed at determining susceptibility of *P. shigelloides* isolated from human and environmental samples to popular antibiotics using antibiogram profiling.

Specifically, environmental samples shall include water, soil and seafood.

MATERIALS AND METHODS:

Study location;

The study location was Port Harcourt, Rivers State Nigeria. Human samples were collected from three strategic hospitals in Port Harcourt at coordinates 4.8156º N, 7.0498º E. All environmental samples including soil, water and seafood were randomly collected in Port Harcourt.

Sample collection:

A total of 300 stool samples were collected at random from humans across the hospitals studied. Environmental samples comprised 160 well-water samples, 50 soil samples from gutters, ponds, rivers, farms and streams. Similarly, 50 seafood samples including *Periophthalmus* sp, *Sardinella* sp and *Penaeus* were purchased in Port Harcourt Metropolis. All environmental samples were collected in sterile bottles and taken to the Microbiology laboratory, at the Rivers State University. Collection of samples was done according to the recommendations of the American Public Health Association (APHA) (16).

Isolation and Identification of *P. shigelloides:*

Isolation and morphological identification of test organism was done on MacConkey and Deoxycholate citrate agar as previously described (17). Freshly collected diarrheal stool samples were plated onto media plates and incubated at 37ºC for 24 h as in (5). Similarly, environmental samples were plated out and incubated accordingly. The suspected colonies were subjected to various biochemical tests for confirmation (18-22).

Antimicrobial Susceptibility Testing:

The susceptibility of isolates was done using the Kirby Bauer Disk diffusion Technique in accordance with the NCCLS procedure as previously reported (14). Commercially available antibiotic disks were obtained and used (Oxoid Code 1788E). A total of eight (8) antibiotics; consisting of Nitrofurantoin (F) (200mg), Nalidixic-acid (NA) (30 mg) Colistin-sulphate (CT) (10 mg), Co-trimoxazole (SXT) (25mg), Sulphonamide (S_3) (30mg), Tetracycline (TE) (50 mg), Ampicillin (Amp) (25 mg) and Streptomycin(S) (25 mg), were tested. Isolates were plated and incubated at 37 ºC for 24 h for antibiogram studies as described (33). All isolates were subjected to the antibiotics disk and sensitivity measured in percentage with relation to the number of isolates that were sensitive to a given antibiotic.

Multiple Antibiotic Resistance (MAR) index was calculated and interpreted as previously explained (34), using

$$\frac{a}{b}$$

Where "a" is number of antibiotics to which the isolates were resistant and "b" is total antibiotics studied.

Data analysis

All statistical analysis was performed using Microsoft Excel. Test for significance was done using chi square and percentages (%) to analyze the data obtained.

Results

A summary of result of biochemical tests on all the isolates obtained from the different sources are shown in Table 1.

Table 1: Biochemical characterization of *P. shigelloides* isolates

Test	***Plesiomonas shigelloides* (39 isolates)**		
	No. +ve Positive	No. -ve after 3 days of incubation	No. negative
Gram's Reaction	Gram negative rods	0	0
Oxidase	39	0	0
Catalase	39	0	0
Motility	39	0	0
Indole	39	0	0
Starch hydrolysis	0	0	39
Gelatin liquefaction	0	0	39
Methyl red	39	0	0
Voges-Proskauer	0	0	0
Citrate Utilization	0	0	39
Urease	0	0	39
Growth in 7.5% NaCl	0	0	39
O/F test	Fermentative	0	0
Hydrogen sulphide production	0	0	39
Gas in glucose	0	0	39

Sucrose	0	0	39
Lactose	37	2	0
Fructose	0	6	31
Maltose	39	0	0
Mannitol	0	0	39
Arabinose	0	0	39
Raffinose	0	0	39
Xylose	0	0	39
Salicin	0	2	37
Inositol	39	0	0

Overall, human samples were more sensitive to most antibiotic (Table 2). The drug sensitivity pattern of the 25 isolates is as shown in Table 2. Thirty nine isolates, from various samples, were randomly tested for sensitivity to eight known antimicrobial agents. All isolates were subjected to all antibiotics and sensitivity/resistance measured in percentage. All the human isolates were 100 % sensitive to, Colistin-sulphate, Nalidixic acid, Nitrofurantoin and Tetracycline. Further co-trimoxazole and sulphonamide showed high activity against *P. shigelloides* with 80 % and 60 % sensitivity respectively (Table 3). However, the test organisms showed resistance to streptomycin and ampicillin with 0 and 20 % sensitivity respectively in human samples.

Table 2: Correlation of Source of Specimen to the sensitivity of Isolates

Source	Number tested	Antibiotics (concentration)							
		AMP(25 mg)	CT(10 mg)	NA(30 mg)	F(200 mg)	S(25mg)	SXT(25 mg)	TE(50 mg)	S_3(30mg)
Human	5	1(20)	5(100)	5(100)	5(100)	0(0.0)	4(80)	5(100)	3(60)
Seafood	2	1(50)	1(50)	2(100)	2(100)	0(0.0)	1(50)	2(100)	1(50)
Soil	8	0(0.0)	8(100)	8(100)	8(100)	0(0.0)	6(75)	6(75)	4(50)
Well-Water	24	1(4.17)	10(41.67)	24(100)	24(100)	0(0.0)	9(37.5)	11(45.83)	7(29.17)

Legend: Ampicillin (AMP), Colistin-sulphate(CT), Nalidixic-acid(NA), Nitrofurantoin (F), Streptomycin(S), Co-trimoxazole (SXT), Tetracycline (TE) and Sulphonamide (S_3).

The antibiogram results showed that *P. shigelloides* isolates from all sources were consistently susceptible to Nalidixic acid and Nitrofurantoin. Both were most (100%) effective against *P. shigelloides*. Their percentage sensitivities for the different drugs are presented in Tables 2 and 3. One of the isolates from seafood was found to be resistant to Colistin sulphate. Resistance to sulphonamide and co-trimoxazole was exhibited by most isolates (Table 3). All isolates were found to be 100% resistant to streptomycin.

Table 3: Susceptibility pattern of *P. shigelloides* isolates obtained during the study.

Antibiotic	Concentration (mg)	No. of isolates tested	Susceptible	Intermediate	Resistant
Ampicillin	25	39	3(7.68)	8(20.48)	26 (66.56)
Colistin sulphate	10	39	24(61.44)	7(17.92)	8(20.48)
Co-trimoxazole	25	39	15(38.4)	7(17.92)	22(56.32)
Nalidixic acid	30	39	39(100)	0(0.00)	0(0.00)
Nitrofurantoin	200	39	39(100)	0(0.00)	0(0.00)
Streptomycin	25	39	0(0.00)	0(0.00)	39(100)
Sulphonamide	30	39	20(51.2)	10(25.6)	9(23.04)
Tetracycline	50	39	23(58.9)	8(20.48)	8(20.48)

Multiple antibiotic resistance (MAR) index calculation was summarised in Table 4 and showed that many of the isolated exhibited multiple resistance to many of the antibiotics studied.

Table 4: Multiple Antimicrobial Resistance (MAR) index of all *P. shigelloides* isolates studied.

MAR Index	Number Tested
0.1	1(0.03)
0.2	0(0.00)
0.3	0(0.00)
0.4	0(0.00)
0.5	0(0.00)
0.6	4(10.26)
0.7	0(0.00)
0.8	18(46.15)
0.9	7(17.9)
1.13	9(23.1)

Note: MAR index >0.2 suggests their origin from a high risk source of contamination where antibiotics are often used.

Based on results from the susceptibility testing, isolates were grouped into six distinct biotypes as in Table 5.

Table 5: Biotyping of *P. shigelloides* isolates by their drug sensitivity pattern.

Biotype	No. of isolates	Drug sensitivity					Species
		AMP	CT	SXT	S_3	TE	
B	5	+	+	+	+	+	*P. shigelloides*
A	15	-	+	+	+	+	*P. shigelloides*
F	7	-	+	+	-	-	*P. shigelloides*
C	6	-	+	-	-	+	*P. shigelloides*
D	3	-	+	-	+	-	*P. shigelloides*
E	3	-	-	-	-	-	*P. shigelloides*

DISCUSSION:

Previous studies (23) reported all strains susceptible to streptomycin. However, Stock and Wiedemann (15) demonstrated a uniform resistance of all strains studied to streptomycin as in the present study. This study showed minimal susceptibility of isolates from humans, water and fish to ampicillin. This finding is in tandem with previous studies (24). Conversely, some studies have reported susceptibility of *P. shigelloides* to ampicillin. The present study showed a sensitivity of 7.68% of the isolates tested. This finding question the use of ampicillin as a selective agent for the isolation of *P. shigelloides* by several investigators (25). Another study (26) however observed that ampicillin containing medium can select for *Pleisomonas* when they are present in very low numbers in the stool and unable to cause diarrhoea in patients. This suggests that the use of ampicillin in selective media is controversial and possibly might have led to erroneous isolation rate in previous studies.

Strains of *P. shigelloides* are said to be consistently susceptible to tetracycline although varying susceptibility have been reported by some workers (24).

In this study, 23 isolates were susceptible to tetracycline which gave a sensitivity of 58.9% of the isolates tested. A sensitivity of 61.44% and 38.4% was recorded for Colistin sulphate and Co-trimoxazole respectively. Sources of isolation appeared to influence the susceptibility of the *P. shigelloides* to a particular antimicrobial agent. For instance, all human isolates seemed to be more susceptible to the antimicrobial agents studied and showed possible pattern of antibiogram. All human isolates were uniformly susceptible to tetracycline, Colistin sulphate, Nalidixic acid and Nitrofurantoin but were resistant to streptomycin. Varying susceptibility was exhibited to co-trimoazole, sulphonamide and ampicillin.

The higher sensitivity recorded in human samples is a great relief considering the prevalence and increase in the menace of drug antibiotic resistance. The greater drug resistance recorded in environmental samples is worrisome and calls for improved environmental practices. The environment is constantly at the receiving end of numerous anthropogenic activities. These could enhance drug resistance. Studies have highlighted hotspots that lead to increased drug

resistance in the environment to include discharge of untreated hospital waste as well as untreated municipal wastes (26, 27). Further discharge of effluents into waters, sewage and animal wastes used as agricultural manure have also been highlighted. All these have turned environment into a reservoir for enormous Antibiotic Resistant Genes and has made for constant horizontal gene transfer (12, 28). Horizontal gene transfer has increased the menace of antibiotic resistance globally. The phenomenon of horizontal gene transfer thus challenges the said relief hinged on high susceptibility of human *P. shigelloides* isolates. This is particularly challenging considering ingestion of sea food and constant human contact with other environmental samples. This raises some concern since water is a major human infection route for *P. shigelloides* (15).

There is a controversy as to whether *Plesiomonas* should be reported by clinical microbiology laboratories whenever they are isolated from faeces (29). Avison *et al*. (30) suggests that *P. shigelloides* should be reported even in low numbers with antimicrobial susceptibility testing performed only on special request. *Plesiomonas* definitely should be reported when they predominate or when their growth is heavy on non-selective media. This is due to difficulty in interpreting the presence of organisms in low numbers, since they may be commensals with no relationship to diarrhoea. Further, studies suggest that diarrhoea may result from a synergy of several microorganisms (1). Holmberg and Farmer (31) also reported that because *P. shigelloides* usually caused mild gastroenteritis in initially healthy persons, such patients do not routinely require treatment with antimicrobial agents but replacement of lost fluid and electrolytes in diarrhoeal stool should be the basis of therapy (32). This would help alleviate the indiscriminate use of antibiotics in cases where the diarrhoea might be self-limiting.

Appreciable Multiple Antibiotic Resistance (MAR) indices were recorded in the present study. A MAR index greater than 0.2 was recorded for 97.1% of all isolates tested (34). This is inactive of a high risk source of contamination and an environment where antibiotic if often used. The multiple antibiotic resistant (MAR) gene is responsible for multiple antibiotic resistance in organisms (34). In Gram negative bacteria such as *P. shigelloides*, MAR is probably due to the presence of various antibiotic resistance genes (35). The mutation and overexpression of these genes lead to AMR because of several factors (36). Some of these factors leading to multiple drug resistance may be linked to the sample sources. Sample sources have been exposed to varying circumstances, explaining their susceptibility and resistance alike.

There are limitations of this method of antibiotyping because of possible development of plasmid associated resistance. However, in the absence of other methods used in antibiotyping this method could be used.

CONCLUSION:

The present study reveals an appreciable sensitivity in strains of *P. shigelloides* isolated from human samples with a worrisome level of drug resistance in environmental isolates. This is against the increasing significance of this organism in human infections. The need for isolation of more strains of *P. shigelloides* from different geographical areas is high now than ever. This will aid in determining their relative importance in acute diarrhoeal disease. This will consider important attributes such as drug resistance, enzyme elaboration and toxigenicity. These may differ from one environment to another.

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