Antibiotic Susceptibility Profiles of Enteric Bacterial Isolates from Dumpsite Utisols and Water Sources in a Rural Community in Cross River State, Southern Nigeria.

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Abstract: A survey was conducted to establish the effects of bacterial contamination from dumpsite effluents on utisol and water and the antimicrobial susceptibility profiles of the bacterial isolates. A total of 504 each of soil and water samples from different locations were sampled between the months of May and November, 2009. *Proteus* sp(70.24%), *Pseudomonas* sp(59.13%), *Bacillus* sp(58.33%), *Escherichia coli* (58.33%), *Campylobacter* sp(45.63%), *Klebsiella* sp(35.12%), *Shigella* sp(30.96%), *Salmonella* sp(27.98%), *Aeromonas* sp (27.98%) and *Vibrio cholerae* (10.91%) were isolated from polluted utisols, while *Bacillus* sp (86.51%), *Pseudomonas* sp (71.23%), *Escherichia coli* (60.71%), *Aeromonas* sp (52.58%), *Salmonella* sp (47.02%), *Klebsiella* (26.19%) and *Vibrio cholerae* (13.10%) were isolated from various water sources. The prevalence of the bacterial species in the two environmental sources differed significantly ($P<0.05$). All isolates were resistant to Gentamicin, Chloramphenicol and Amikacin, while low resistance values were recorded in Erythromycin (25%) and Nalidixic acid (37.50%). Adequate treatment of dumpsite effluents and the use of Erythromycin and Nalidixic acid as therapeutic measures are recommended to reduce possible health hazards.
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1. Introduction

The various uses of water for drinking, bathing, washing and cooking are well known. Water meant for human consumption should be free from pollution, and should be safe and acceptable. Indeed the microbial quality of potable water should not exceed limits specified in the water quality guideline (APHA, 1998). However, the microbial quality of water in several rural Nigerian communities has been reported to be poor, unsafe and not acceptable for human consumption (Obi *et al*, 2004). Enteric bacterial pathogens have been shown to thrive for long periods in water in spite of a large number of antagonistic populations (Hoge *et al*, 1989). These pathogens are variously incriminated in cases of diarrhea which in turn accounts for a substantial degree of morbidity and mortality in different age groups worldwide (Black, 1993 and Obi *et al*, 1997). Water sources in Akansoko are recipients of heavy microbial load through anthropogenic activities. Akansoko is a community located on the outskirts of Calabar and situated strategically on the banks of one of the tributaries of the lower Cross River. Its immediate neighborhood serves as one of the numerous dumpsites for municipal wastes.

Isolation of pathogens from sources connotes a serious public health risk to consumers. This risk is further exacerbated by the widely reported cases of resistance of enteric bacterial pathogens to several antibiotics (Hoge *et al*., 1998; Ash *et al*., 2002). For example, in 1984, 82% of *Campylobacter* strains from Lagos, Nigeria, were sensitive to erythromycin and 10years later only 70.8% were sensitive (Coker and Adafaso, 1994). In Thailand, ciprofloxacin resistance among *Campylobacter* species increased from 0% before 1991 t0 84% in 1995 (Hoge *et al*., 1998). In the United States, several rivers were reported to be reservoirs of antibiotic resistant bacteria (Ash *et al.*, 2002). Even though antibiotic resistance is common, antibiotics are still used in the management of diarrhea. Antibiotics shorten the duration of diarrhea, decrease stool output, and may mitigate complications (Black, 1993.)

This study was therefore aimed at evaluating the antibiogram of enteric bacterial isolates from water sources and dumpsites effluents in Akansoko community using commonly used antibiotics scheduled for diarrhea cases.

2. Materials and Methods

2.1 Study Area.

The study area is Akansoko village, located approx. 15 km Southeast of Calabar, the Cross River State capital, Nigeria. It is situated between latitudes $4^{o}30^{/}$ and $5^{o}00^{/}$N and longitude $5^{o}15^{1}$ and $8^{o}45^{1/}$E. Major sources of water supply are dug-out wells, streams and slow flowing portions of great Kwa River

estuary, which forms part of the lower Cross River estuarine system (Eja *et al*., 2003).

2.2. Sample Collection

Sampling was done thrice weekly from May to November 2007.

Water samples were collected from two sources, two stream sources and an estuary located within the study area. Duplicated samples were collected in sterile one liter Nalgene containers and transported in ice box at 4^{o}C to the microbiology laboratory within 6h for analysis.

Effluent contaminated soils from dumpsites were collected from 6 stations within the study community. Each duplicate sample was collected in sterile polyethene bags and transported in an ice-cold container to the microbiology laboratory, Cross River University of Technology for analysis within 8h.

2.3 Sample Analysis

The organisms were isolated using standard methods (APHA, 1998)

For the isolation of *Campylobacter*, Skirrows and Butchers' medium (LAB-M) was used. Blood Agar plates were incubated at 42^{o}C under microaerophilic conditions by placing in candle jar for 72h. Colonies were considered to be *Campylobacter* if they were S-shaped, Gram negative, motile and oxidase positive .

For the isolation of *Salmonella* and *Shigella* 1ml water sample and 10^{-1} soil dilution were inoculated in 9ml selenite-F-broth and incubated for 18-24h at 37^{o}C for enrichment. The enriched samples was plated on *Salmonella-Shigella* Agar (Oxoid) and incubated for 48h at 37^{o}C. Small colourless colonies were subcultured on nutrient agar slants and identified using methods described by Cowan and Steel (1985).

For the identification of *Vibrio cholerae*, 1.0ml of water sample and 10^{-1} soil dilution were enriched in alkaline peptone water (pH 8.6) and incubated at 37^{o}C for 8hrs. Several loopfuls of the peptone water culture (taken from the surface) were streaked on Thiosulphate Citrate Bile Salt Sucrose (TCBS) Agar (Difco) and incubated at 37^{o}C for 24h. Yellow colonies were subcultured on nutrient agar slants to produce pure cultures. Microscopic and biochemical tests were used to identify *Vibrio cholerae* as described by Cowan and Steel (1985).

Other enterobacteria were identified by culturing samples on MacConkey agar (Oxoid) and characteristic colonies subcultured on nutrient agar slants for further biochemical tests.

Non-enterobacterial species were identified by culturing on nutrient agar and subsequent biochemical tests carried out as described by Cowan and Steel(1985).

2.4. Antibiotic Susceptibility Testing

Pure isolates were cultured for antibiotic susceptibility assessment using the disc diffusion method (Obi *et al.,* 2004). Antibiotic-impregnated discs were placed on Mueller Hinton Agar (Difco) and incubated at 37^{o}C for 24h. Zones of inhibition were measured and compared with standard values. Antibiotics in the panel included Tetracycline(30µg),Gentamycin(120µg),Erythromycin(10µg), Ampicillin(10µg), Chloramphenicol(30µg), Nalidixic acid(30µg) and Amikacin(30µg) with concentrations as recommended by the National Committee for Clinical Laboratory Standards(NCCLS, 1996).

3. Results

Bacterial isolates obtained from soils at various dumpsites in the study community are shown in the Table 1.0. *Proteus* sp, *Bacillus* sp, *Pseudomonas* sp, and *Escherichia coli* were isolated from more than 50% of the total samples. The prevalence of the various bacterial species differed significantly ($P<0.05$) throughout the sampling period. All species were isolated in the various sampling stations.

Table 2.0 shows the occurrence of bacterial isolates from water sources in the community. *Bacillus* sp, *Pseudomonas* sp, *Escherichia coli* and *Aeromonas* sp recorded prevalence values higher than 50% in all samples analyzed. The prevalence of all species were also observed to differ significantly ($P<0.05$). Enteric pathogens such as *Vibrio cholerae*, *Salmonella* sp, *Escherichia coli* and *Shigella* sp had higher prevalence values in samples from streams and estuary.

Antibiogram results of the various isolates as presented in Table 3.0 show multidrug resistance by all isolates. Resistance of isolates (100%) was observed on Gentamicin, Chloramphenicol and Amikacin. Similarly, 87.5% and 75% of isolates were resistant to tetracycline and Ampicilin, respectively. However, Erythromycin and Nalidixic acid displayed better performing index with 25% and 37.5% isolates resistance, respectively.

Table 1.0: Occurrence of bacterial isolates from dumpsites soils at various sampling stations

Bacterial Isolates	Sampling station 1 N=84(%)	Sampling station 2 N=84(%)	Sampling station 3 N=84(%)	Sampling station 4 N=84(%)	Sampling station 5 N=84(%)	Sampling station 6 N=84(%)	Total Frequency N=504(%)
Bacillus sp	63(75.00)	65(77.38)	82(97.62)	21(25.00)	43(51.19)	20(23.81)	294(58.33)
V.cholerae	10(11.90)	17(20.24)	6(7.14)	12(14.29)	2(2.38)	8(9.52)	55(10.91)
Aeromonas sp	38(45.24)	53(63.10)	14(16.67)	10(11.90)	7(8.33)	11(13.10)	133(26.39)
Salmonella sp	41(48.81)	30(35.71)	34(40.48)	10(11.90)	17(20.24)	9(10.71)	141(27.98)
Shigella sp	49(82.14)	34(40.48)	36(42.86)	15(17.86)	10(11.90)	12(14.29)	156(30.95)
E. coli	69(82.14)	73(86.90)	58(69.05)	13(15.48)	61(72.62)	20(23.81)	294(58.33)
Campylobacter sp	59(70.24)	62(73.81)	31(36.90)	17(20.24)	28(33.33)	33(39.29)	230(45.63)
Pseudomonas sp	47(55.95)	52(61.90)	75(89.29)	61(72.62)	32(38.10)	29(34.52)	298(59.13)
Klebsiella sp	27(32.14)	34(40.48)	51(60.71)	40(47.62)	11(13.10)	14(16.67)	177(35.12)
Proteus sp	82(97.62)	66(78.57)	77(91.67)	48(57.14)	53(63.10)	28(33.33)	354(70.24)

Total $P<0.05$

N=Total No of samples analyzed

Table 2.0: Occurrence of bacterial isolates from water sources at various sampling locations

Bacterial Isolates	Well 1 N=84(%)	Well N=84(%)	Well 3 N=84(%)	Stream 1 N=84(%)	Stream 2 N=84(%)	Estuary N=84(%)	Total Frequency N=504(%)
Bacillus sp	71(84.52)	69(82.14)	65(77.38)	75(89.29)	67(79.76)	79(94.05)	436(86.51)
V. cholerae	3(3.57)	11(13.10)	5(5.95)	18(21.43)	13(15.48)	16(19.05)	66(13.10)
Aeromonas sp	38(45.24)	62(73.81)	13(15.48)	17 (20.24)	54(62.29)	81(96.43)	265(52.58)
Salmonella sp	67(79.76)	24(28.57)	41(48.81)	35(41.67)	21(25.00)	49(58.33)	237(47.02)
Shigella sp	9(10.71)	24(28.57)	12(14.29)	20(23.81)	28(33.33)	16(19.05)	109(21.63)
E. coli	44(52.38)	52(61.90)	22(26.19)	61(72.62)	69(82.14)	58(69.05)	306(60.71)
Campylobacter sp	12(14.29)	19(22.62)	4(4.76)	9(10.71)	17(20.24)	23(27.38)	84(16.67)
Pseudomonas sp	56(66.67)	63(75.00)	41(48.81)	60(71.43)	65(77.38)	74(88.10)	359(71.23)
Klebsiella sp	11(13.10)	27(32.14)	14(16.67)	38(45.24)	12(14.29)	30(35.71)	132(26.19)
Proteus sp	7(8.33)	2(25.00)	4(4.76)	31(36.90)	29(34.52)	(18(21.43)	91(18.06)

Total $P < 0.05$

N = Total No of samples analyzed

Table 3.0: Antibiotic susceptibility profile of major pathogenic bacterial isolates from dumpsites and water sources

	Antibiotics susceptibility (Zone of inhibition in mm)						
Isolates	TET	GEN	ERT	AMP	CLP	NAL	AMK
V. cholerae	R(16)	R(18)	R(16)	S(20)	R(16)	S(20)	R(17)
Aeromonas sp.	R(14)	R(10)	R(12)	R(8)	R(11)	R(16)	R(11)
Salmonella sp.	R(18)	R(16)	S(28)	R(11)	R(10)	S(26)	R(16)
Shigella sp	R(16)	R(10)	S(21)	R(10)	R(13)	S(20)	R(18)
E. coli	R(16)	R(9)	S(26)	R(10)	R(12)	S(29)	R(14)
Campylobacter sp.	R(17)	R(18)	S(27)	R(19)	R(10)	S(26)	R(19)
Pseudomonas sp.	R(16)	R(11)	S(25)	R(9)	R(10)	R(12)	R(13)
Klebsiella sp.	S(27)	R(12)	S(20)	S(26)	R(16)	R(11)	R(9)
Total Resistance (%)	7(87.50)	8(100.00)	2(25.00)	6(75.00)	8(100.00)	3(37.50)	8(100.00)

R = Resistant, S = Susceptible, TET= Tetracycline, GEN = Gentamicin, ERT= Erythromycin, AMP = Ampicillin, CLP= Chiliramphenicol, NAL= Nalidixic acid, AMK =Amikacin

4.1 Discussion

Many anthropogenic activities have been known to be major causes of pollution of soil and water sources. The community under study relies on water sources which are devoid of treatment for their domestic needs. Most of these sources are situated at proximal distances to various dumpsites.

Bacterial species isolated from soils at dumpsites showed high prevalence of *Proteus* sp (70.21%), *Pseudomonas* sp (59.13%), *Bacillus* sp (58.33%) and *Escherichia coli* (58.37%). Other major enteric pathogens such as *Salmonella* sp (47.02%), *Shigella* sp (21.63%) and *Vibrio cholerae* (13.10%) were also isolated. The presence of these pathogens may be due to domestic wastes which usually form the main component of wastes at the dumpsites. Udo and Nfongeh (2005) working in Adim community, Cross River State, Nigeria isolated *Vibrio cholerae* (14.7%), *Salmonella paratyphi* (12.5%), *Salmonella typhimurium* (8.8%) and *Shigella sonnei* (6.3%) as major enteropathogens associated with diarrhea cases. The isolation of these pathogens at dumpsites directly confirms the presence of faecal wastes at various dumpsite since it was common practice to dump human excreta in the sites which could also be used as latrines at night.

Similarly, *Bacillus* sp (86.51%), *Pseudomonas* sp (71.23%), *Escherichia coli* (60.71%), *Aeromonas* sp (52.58%) and *Salmonella* sp (47.02%) were the major bacterial species isolated from water sources proximal to dumpsites. These species are directly related to those isolated from soils at dumpsites. This similarity suggests possible percolation of pathogens in dumpsite effluents through the soil into water sources. The residents used the sampled water sources as their main source of water supply for domestic needs.

Antibiograms of the major enteric pathogens isolated reveal resistance to preferred drugs. All isolates were resistant to Gentamicin, Chloramphenical and Amikacin while Erthromycin (25%) and Nalidixic acid (37.50%) had low resistance percentages for the isolates. The fact that several bacterial species are known to be resistant to a wide array of antibiotics was confirmed by Aseffa *et al.,*(1997). The marked resistance of strains of *Salmonella* and *Shigella* to Ampicillin and Chloramphenicol as shown in the present study agrees with the findings of Ash *et al.*, (2002) working on rivers in the United States and probably accounts for the major outbreaks of salmonellosis and shigellosis worldwide. Similarities in antibiograms among isolates from both environmental sources indicate a possible infiltration of pathogens from dumpsite effluents to water sources. Persistent multiple drug resistance of most isolates to appropriate drugs of choice are of great public health concern and calls for periodic monitoring of antibiograms to detect possible changing patterns.

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