

## Occurrence of drug-resistant bacteria in communal well water around Port Harcourt, Nigeria

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### SUMMARY

A total of 108 raw water samples was collected from 36 wells at nine shanty settlements around Port Harcourt, Nigeria, over a period of 7 months. Samples were analysed for their bacteriological quality. Selected bacterial strains isolated from the samples were tested for their susceptibility to ten commonly used antibiotics. The organisms isolated include *Pseudomonas* spp., *Klebsiella* spp., *Staphylococcus* spp., *Proteus* spp., *Enterococcus faecalis*, *Aeromonas* spp., *Escherichia coli*, *Chromobacterium* spp., *Flavobacterium* spp., and *Serratia* spp.

Out of 300 strains tested, 23 (6.9%) were susceptible to all the antibiotics, 277 (92.3%) were resistant to at least one antibiotic and 232 (77.3%) were resistant to two or more antibiotics. The epidemiological significance of these results is discussed.

### INTRODUCTION

The presence of coliforms, especially the faecal coliform, is regarded as an index of bacteriological quality of water and food. The faecal coliform along with some other members of the Enterobacteriaceae such as *Salmonella*, *Shigella*, *Klebsiella*, *Proteus*, *Serratia*, *Pasteurella*, *Yersinia* and *Erwina* as well as *Vibrio* and *Pseudomonas* species are known to be involved in the transfer of antibiotic resistance by means of R-factors (Chatterjee & Starr, 1972; Duguid, Marmon & Swain, 1978; Linton, Timoney & Hinton, 1981).

It is now known that the use of antibiotics in medicine and other fields provides an intense selection pressure in favour of micro-organisms possessing resistance genes (Richmond *et al.* 1979; Hinton, Kaukas & Linton, 1986).

Various studies have shown the possibility of transfer of antibiotic resistance from commensal enteric bacteria to pathogenic enteric bacteria and vice versa, (Richmond *et al.* 1979; Linton, Timoney & Hinton, 1981). Such organisms, with multiple resistance to antibiotics, have been isolated in appreciable numbers from rivers and other bodies of water (Al-Jebouri, 1985).

In shanty settlements where inhabitants depend on shallow wells as the only source of water, the presence of antibiotic-resistant organisms could be hazardous.

Some other investigators have reported on *Staphylococcus aureus* and *E. coli* (e.g. Antai, 1987). The present study was designed to determine the bacterial flora of communal wells and the level of resistance of the enteric bacteria and other organisms from these sources to commonly used antimicrobial agents.

#### MATERIALS AND METHODS

##### *Sample collection*

One hundred and eight samples of raw water were collected from 36 randomly selected wells, used as sources of communal water supply, in nine shanty settlements around Port Harcourt, Nigeria. The water was first drawn in the container used by the inhabitants and then transferred aseptically into autoclaved sampling bottles. Each well was sampled three times within 7 months. All the shanty settlements are at water-front 0.5 to 2 m above mean sea level. Wells are 3–5 m deep with the edges elevated from 10–60 cm above the ground. Each well serves 400–930 persons.

##### *Demographic survey*

The number of persons served per well was determined by obtaining the total number of persons in the settlement and dividing this number by the number of wells identified.

The survey included a determination of whether the wells were cemented. The sanitary condition of the environment, in particular, the system of disposal of human wastes, was also determined.

##### *Bacterial isolation and identification procedures*

Total bacterial counts were made on standard plate count agar (Difco) incubated at 35 °C for 48 h. The procedures described by Al-Jebouri (1985) for the isolation of *Escherichia coli* and other coliforms were used. All the samples were examined for total plate counts, as well as faecal and total coliform counts.

A total of 309 colonies was subcultured on MacConkey Agar (Difco) for purity and identification. Bacteria were identified to their genera using colonial morphology, Gram reaction, and biochemical tests (Edward & Ewing, 1972; Cowan, 1974).

##### *Antibiotic susceptibility testing*

The antibiotic susceptibility patterns of 300 strains isolated were determined on Diagnostic Sensitivity Test (DST) agar (Oxoid CM 261). Both single disks and Multodisks (Oxoid U4) were used according to the techniques described by Johnston, Bruce & Hill (1983) and Al-Jebouri & Al-Meshhadani (1985). The Multodisk contained ( $\mu\text{g}$ ): ampicillin, 25; carbenicillin, 100; cephaloridine, 25; colistin sulphate, 10; co-trimoxazole, 25; gentamicin, 10; sulphafurazole, 500; tetracycline, 50. The single disks used contained ( $\mu\text{g}$ ): chloramphenicol, 10; streptomycin, 25.

Table 1. *Distribution of sampling stations at shanty settlements around Port Harcourt*

Station no.	Location of well	Wells sampled per station	Status of well at sampling	Persons served per well
1	Baptist Waterside	4	Shallow, covered edged 25 cm above ground	650 ± 60
2	Bundu Waterside	4	Shallow, covered 10–20 cm above ground	470 ± 80
3	Creek Road	4	Shallow, covered 30 cm above ground	830 ± 20
4	Elechi Beach, Waterside	4	Shallow, covered 30 cm above ground	460 ± 13
5	Enugu Waterside	4	Shallow, some uncovered ground	810 ± 28
6	NEPA Waterside	5	Shallow, covered 20–30 cm above ground	840 ± 28
7	Ogu Waterside	4	Shallow, covered 30–40 cm above ground	730 ± 20
8	Okrika Waterside	3	Shallow, not covered 30–40 cm above ground	480 ± 20
9	Uruala Waterside	4	Shallow, not covered 20–40 cm above ground	720 ± 50

## RESULTS

*Distribution of sampling stations*

The location of the shanty settlements visited for this study and the distribution and description of the wells are shown in Table 1. It was found that the walls of some of the wells were not cemented. Toilet facilities were either of the bucket (pail) system or of the system of wooden jetties culminating in roofed cubicles from which people defaecated directly into the surrounding estuarine water.

*Bacterial flora of shallow well water*

The total plate counts together with the total and faecal coliform counts are given in Table 2. The total plate count values appear high for most of the wells ( $10^3$ – $10^5$  c.f.u. ml<sup>-1</sup>). Coliforms were detected in all the wells except one (No. 3). Faecal coliform count was high in five stations and relatively low in Station No. 7 (2 c.f.u./100 ml). None was detected from wells in three stations (Table 2). The different organisms isolated from all the wells are shown in Table 3. *Pseudomonas* spp. were the most frequently encountered, followed by *Klebsiella* spp. The major coliform organisms constituted 28.3% of the isolates.

Table 2. *Bacterial population in well water at shanty settlements*

Station	SPC/ml	TC-MPN/100 ml	EC-MPN/100 ml
1	$4.2 \times 10^4$ (1.3–11.0 $\times 10^4$ )	$1.4 \times 10^3$ (2.2–2.4 $\times 10^3$ )	6 (2.0–11)
2	$1.6 \times 10^5$ (2.0–2.4 $\times 10^5$ )	1 (0–2)	0
3	$4.5 \times 10^4$ (0.1–12.8 $\times 10^4$ )	0	0
4	$3.8 \times 10^4$ (1.5–5.1 $\times 10^4$ )	$1.1 \times 10^3$ (0.2–2.4 $\times 10^3$ )	5 (0–11)
5	$4.5 \times 10^3$ (2.5–6.5 $\times 10^3$ )	$6.5 \times 10$ (0–1.3 $\times 10^2$ )	2 (0–3)
6	$3.3 \times 10^4$ (1.2–5.4 $\times 10^4$ )	3 (0–6)	0
7	$8.5 \times 10^4$ (1.3–40 $\times 10^4$ )	$8.7 \times 10^2$ (0.1–1.3 $\times 10^3$ )	1 (0–2)
8	$7.2 \times 10^4$ (5.4–9.0 $\times 10^4$ )	$1.6 \times 10^3$ (1.1 $\times 10^3$ –1.9 $\times 10^3$ )	5 (2–11)
9	$4.6 \times 10^4$ (7.0 $\times 10^3$ –1.2 $\times 10^5$ )	$5.1 \times 10^2$ (2 $\times 10$ –1.6 $\times 10^3$ )	4 (0–9)

Values in parentheses represent the range; EC, *Escherichia coli*; MPN, most probable number; SPC, standard plate count; TC, total coliforms; results are the mean values of three sets of samples collected from each of the wells in the study stations.

Table 3. *Range of organisms isolated from shallow well water of shanty settlements around Port Harcourt*

Organism	No. isolated	Percentage occurrence
<i>Pseudomonas</i> spp.	60	19.4
<i>Klebsiella</i> spp.	45	14.6
<i>Staphylococcus</i> spp.	35	11.3
<i>Acinetobacter</i> spp.	30	9.7
<i>Alcaligenes</i> spp.	24	7.8
<i>Citrobacter</i> spp.	17	5.5
<i>Enterobacter</i> spp.	14	4.5
<i>Aeromonas</i> spp.	14	4.5
<i>Proteus</i> spp.	13	4.2
Unidentified spp.	11	3.6
<i>E. coli</i>	9	2.9
<i>Candida</i> spp.	9	2.9
<i>Serratia</i> spp.	7	2.3
<i>Bacillus</i> spp.	7	2.3
<i>Flavobacterium</i> spp.	6	1.9
<i>Enterococcus faecalis</i>	5	1.6
<i>Chromobacterium</i> spp.	3	0.97
Total	309	100%

#### *Antibiotic resistance among isolates*

The level of antibiotic resistance among the isolates was high for sulphafurazole (73%), cephaloridine (60%), ampicillin (46.3%) and co-trimoxazole (30.7%). Resistance to each of the other drugs was less than 25% (Table 4). All the isolates were susceptible to gentamicin, except for a few strains of *Acinetobacter* spp., *Alcaligenes* spp., and *Flavobacterium* spp. (Table 4). *Candida* spp. isolated were not tested for antibiotic susceptibility as they are not bacteria.

Only a small proportion (6.9%) of the strains tested was susceptible to all the antibiotics. Most of them (277 strains or 92.3%) were resistant to at least one antibiotic, with a substantial number (232 strains or 77.3%) being resistant to two or more (Table 5).

Table 4. Levels of resistance to individual antibiotics among bacteria from well water in shanty settlements around Port Harcourt

Organism	No. of strains	No. of strains resistant to									
		AMP	CAR	CEP	CHL	COL	COT	GEN	STR	SUL	TET
<i>Pseudomonas</i> spp.	60	48	23	57	40	14	35	—	25	55	6
<i>Klebsiella</i> spp.	45	15	9	36	10	5	20	—	3	43	18
<i>Staphylococcus</i> spp.	35	9	2	10	5	1	2	—	—	22	5
<i>Acinetobacter</i> spp.	30	1	1	13	1	—	12	4	1	25	—
<i>Alcaligenes</i> spp.	24	13	8	18	4	6	5	7	1	10	—
<i>Citrobacter</i> spp.	17	5	3	4	4	—	7	—	—	10	2
<i>Enterobacter</i> spp.	14	10	1	13	1	2	—	—	1	12	1
<i>Aeromonas</i> spp.	14	10	7	7	2	—	2	—	1	14	—
<i>Proteus</i> spp.	13	6	7	7	4	4	1	—	3	13	3
Unidentified spp.	11	9	9	9	1	5	1	—	—	9	—
<i>Escherichia coli</i>	9	3	—	3	1	3	—	—	—	6	5
<i>Bacillus</i> spp.	7	2	1	4	—	2	—	2	1	—	1
<i>Serratia</i> spp.	7	3	3	4	—	1	—	—	—	3	—
<i>Flavobacterium</i> spp.	6	3	3	3	—	4	—	—	—	2	—
<i>Enterococcus faecalis</i>	5	—	—	—	—	4	3	—	—	1	—
<i>Chromobacterium</i> spp.	3	2	1	2	—	1	1	—	—	—	—
Total*	300	139	78	180	74	49	92	16	41	219	41
Resistance (%)		46.3	26	60	24.7	16.3	30.7	5.3	13.7	73	13.7

\* Nine *Candida* spp. were not tested; the antibiotics used were ( $\mu\text{g}$ ): AMP, ampicillin (25); CAR, carbenicillin (100); CEP, cephaloridine (25); CHL, chloramphenicol (10); COL, colistin sulphate (10); COT, co-trimoxazole (25); GEN, gentamicin (10); STR, streptomycin (25); SUL, sulphafurazole (500); TET, tetracycline (50).

Table 5. Incidence of antibiotic resistance among bacteria isolated from shallow well water at shanty settlements around Port Harcourt

Organisms	No. of isolates	No. susceptible to all antibiotics tested	No. (%) resistant to one antibiotic	No. (%) resistant to two antibiotics
<i>Pseudomonas</i> spp.	60	—	60 (100)	59 (98)
<i>Klebsiella</i> spp.	45	1	44 (98)	41 (91)
<i>Staphylococcus</i> spp.	35	8	27 (77)	17 (49)
<i>Acinetobacter</i> spp.	30	4	26 (87)	17 (57)
<i>Alcaligenes</i> spp.	24	3	21 (88)	21 (88)
<i>Citrobacter</i> spp.	17	1	16 (94)	12 (71)
<i>Aeromonas</i> spp.	14	—	14 (100)	13 (99)
<i>Enterobacter</i> spp.	14	1	13 (93)	12 (86)
<i>Proteus</i> spp.	13	—	13 (100)	9 (69)
Unidentified spp.	11	—	11 (100)	9 (81)
<i>E. coli</i>	9	1	8 (89)	3 (33)
<i>Bacillus</i> spp.	7	3	4 (57)	2 (29)
<i>Serratia</i> spp.	7	—	7 (100)	5 (71)
<i>Flavobacterium</i> spp.	6	—	6 (100)	5 (83)
<i>Enterococcus faecalis</i>	5	1	4 (80)	4 (80)
<i>Chromobacterium</i> spp.	3	—	3 (100)	3 (100)
Total	300	23 (6.9)	277 (92.3)	232 (77.3)

Numbers in parentheses are percentages.

*Pseudomonas*, *Proteus*, *Aeromonas*, *Serratia*, *Chromobacterium* and *Flavobacterium* species exhibited 100% resistance to one or another of the antibiotics while *Chromobacterium* spp. exhibited 100% resistance to two antibiotics. In nearly every case where an organism exhibited simultaneous resistance to two or more antibiotics, the list of antibiotics included sulphafurazole. Ampicillin, carbenicillin, cephaloridine and chloramphenicol also appeared fairly frequently in the list of antibiotics in instances of multiple resistance to three or more drugs (Table 6).

#### DISCUSSION

The bacteriological quality criteria generally applicable to drinking water supplies to small communities are less than 10 coliforms/100 ml, and less than 2.5 *E. coli* per 100 ml of water. Based on these, only water from three stations (Bundu, Creek Road, and NEPA watersides) meets the international standards for potable water. Water from the other shanty settlement studied may not therefore be suitable for human consumption, without treatment (Havelaar, 1981; Anon., 1983; Report, 1983).

It has been shown that, in some situations, biological contaminants can travel long distances underground without appreciable attenuation by aquifer material (Birden & Cech, 1981). Also, it is known that *E. coli* is rarely found in soil, vegetation or water in the absence of excremental contamination (Report, 1983). The wells from which *E. coli* were recovered in the present study could probably therefore have been exposed to some kind of faecal contamination. Consumption of water contaminated with faecal material has been known to result in outbreaks of bacillary dysentery, enteric fever, cholera, giardiasis, viral

Table 6. Antibiotic resistance pattern among selected bacteria from shallow well water at shanty settlements around Port Harcourt

Organism	Resistance pattern	Frequency*
<i>Pseudomonas</i> spp.	CEP, SUL	4
	AMP, CAR, SUL	4
	CEP, CHL, SUL	4
	AMP, CEP, CHL, COT, SUL	10
	AMP, CAR, CEP, CHL, COT, SUL	10
	AMP, CAR, CEP, COL, STR, SUL	5
	AMP, CEP, CHL, COL, STR, SUL	6
	AMP, CEP, COT, STR, SUL, TET	3
	AMP, CAR, CEP, CHL, COT, STR, SUL	4
	<i>Klebsiella</i> spp.	COT, SUL
CEP, CHL, SUL		3
CEP, SUL, TET		3
AMP, CAR, CEP, SUL		3
CEP, COT, SUL, TET		4
<i>Staphylococcus</i> spp.		SUL
	TET	3
	CEP, SUL	4
	AMP, CHL, SUL	3
<i>Citrobacter</i> spp.	SUL	4
	AMP, CEP, COT	3
	CHL, COT, SUL	3
<i>Enteriobacter</i> spp.	CEP, SUL	4
	AMP, CEP, SUL	6
<i>Proteus</i> spp.	SUL	3
	AMP, CAR, CHL, COT, STR, TET	3
<i>E. coli</i>	TET	5
	AMP, CEP, COL, SUL	3

\* Only resistance patterns occurring  $\geq 3$  times recorded; antibiotics and their concentrations as in Table 4.

gastroenteritis and campylobacter enteritis (Gangarosa *et al.* 1972; Mentzing, 1981). It would appear, therefore, that an estimated population of 28000 persons living at some of the shanty settlements studied may be exposed to such public health hazards.

The level of susceptibility to gentamicin observed among the isolates tested in the present study (94.7%) is comparable to the results of previous investigators (Cooke, 1976; Johnston, Bruce & Hill, 1983; Wray *et al.* 1986). This may in part be because *Staphylococcus* spp. and the Enterobacteriaceae are generally susceptible to gentamicin (Gillett, Wise & Geddes, 1978; Noone, 1978). It may also be because the drug is not used as frequently as other chemotherapeutic agents on account of its nephrotoxic side effects (Baker & Breach, 1980).

Although the level of susceptibility to streptomycin, an aminoglycoside, was somewhat high (only 13.7% of the 300 isolates tested were resistant), it was lower than that to gentamicin, another aminoglycoside (Table 4). This is in close agreement with previous reports that gentamicin is more effective against the coliforms and other Gram-negative bacteria than streptomycin (Noone, 1978).

Unlike the aminoglycosides, however, many of the other antibiotics were not particularly effective against the organisms tested. Most of the isolates (73%),

especially the coliforms were, for instance, resistant to sulphafurazole, a sulphonamide (Table 4). This is similar to the level of resistance among *E. coli* (94%) observed by Linton, Timoney & Hinton (1981), particularly when the level of resistance among *E. coli* and other coliforms in the present study is considered separately.

Whilst the level of resistance to cephaloridine was generally high (60%), resistance was more pronounced among the Gram-negatives than the Gram-positives. This might be attributable to the greater susceptibility of the  $\beta$ -lactam ring of the cephalosporins to  $\beta$ -lactamases elaborated by Gram-negative bacteria than to similar enzymes from *S. aureus*, for example (Barry, 1976).

The levels of resistance to ampicillin and carbenicillin, two penicillins, were relatively low but still appreciable (46.3% and 26%, respectively) (Table 4). This becomes more serious when it is considered that the effect of these two antibiotics on bacteria is similar to that of the cephalosporins (Meyers, Jawetz & Goldfie, 1980) and that all possess  $\beta$ -lactam rings (Barry, 1976). Besides, some of the organisms were simultaneously resistant to all three or at least two of these antibiotics (Table 6).

The results clearly show that there was no drug, among those tested, to which all isolates were susceptible (Table 4). The occurrence of multiply resistant strains of *Pseudomonas*, *Alcaligenes* and *Acinetobacter* spp., among others, in the wells sampled (Table 5) was in agreement with the observations of Armstrong (1981) and Sokari, Ibiebele & Ottih (1981).

On the whole, 92.3% of the organisms tested were resistant to at least one antibiotic while 77.3% were resistant to two or more (Table 5). Such levels of resistance would be attributable, at least in part, to the uncontrolled use of antibiotics and the practice of self-medication common in Nigeria (Antai & Anozie, 1987; Sokari, Ibiebele & Ottih, 1988); for while the use of antibiotics may not cause bacteria to become resistant, the increasing use of the drugs would provide an intense selection pressure in favour of organisms that possess genes coding for drug resistance (Hinton, Kaukas & Linton, 1986).

Linton, Timoney & Hinton (1981) and Al-Jebouri (1985) have pointed out the importance of bacteria acting as a reservoir of plasmids coding for antibiotic resistance. The ingestion of such resistant bacteria by humans could lead to a transfer of drug resistance to the recipient's gut flora and/or to susceptible pathogens by cross infection (Linton, 1977). The existence of multi-resistant bacteria in the wells studied, therefore, constitutes a public health hazard.

It has already been noted that the authorities in Nigeria plan to make antibiotics available to patients only on doctor's prescriptions (Sokari, Ibiebele & Ottih, 1988). Whether such plans, when brought into force, would appreciably reduce the level of drug resistance among bacteria can only be revealed by future studies.

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