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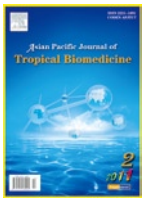

Document heading

Bacterial pathogens and their antimicrobial susceptibility in Otukpo Benue state of Nigeria

Okwori EE*, Nwadioha SI, Nwokedi EOP, Odimayo M, Jombo GTA

Department of Medical Microbiology and Parasitology, College of Health Sciences, Benue State University

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ABSTRACT

Objective: To isolate bacterial pathogens and test for their antibiotic susceptibility. **Methods:** A total of 20000 samples from 9 different clinical sites were processed in the laboratory between 1987 to 2000. The specimens were inoculated on the appropriate media for the isolation of the bacteria. Biochemical and serology tests were carried out on the organisms to confirm the type of bacteria isolated. Antibiotic susceptibility test was also carried out on each of the bacteria isolated. **Results:** A total of 18520 bacteria were isolated from the specimens. The specimens were from nine different clinical sites, *i.e.* wound accounted for 22.84%, urine 31.67%, blood 12.38%, genital 7.70%, sputum 6.81%, stool 6.28%, cerebrospinal fluid 5.98%, aspirates 3.85% and ear/throat swabs were 2.49%. Gram negative bacteria accounted for 76% of isolates. The main species were *Pseudomonas* 2238 (12.08%), *Escherichia coli* (*E. coli*) 2073 (11.19%) and *Staphylococcus aureus* (*S. aureus*) which accounted for 2511 (13.56%) of the total isolates. *S. aureus* showed 70% and 65% resistance to penicillin and ampicillin, respectively. Surprisingly, 40% of the organism was resistant to cloxacillin. *E. coli* showed 47% and 42% resistance to ampicillin and gentamicin, respectively. 49% of *Salmonella typhi* was resistant to chloramphenicol while 37% of *Neisseria meningitidis* was resistant to penicillin. **Conclusions:** The rate of bacteria isolated from the clinical specimens is high and antibiotic sensitivity pattern of the organisms vary from one antibiotic to the other.

1. Introduction

Many diseases that were considered life threatening before World War II are now readily treated with antibiotics and other antimicrobials[1,2]. Before the discovery of penicillin, which initiated the antibiotic era, the prognosis for people with infections diseases, such as bacterial pneumonia, tuberculosis and staphylococcal infections was grim[3]. Physicians were able to identify the causes of the disease, but were generally unable to recommend treatment other than bed rest[4]. Today antimicrobial drugs are widely prescribed. And simple cure for infections is provided. Unfortunately, the over use and misuse of these life-saving drugs, coupled with the bacterial world disability to adapt under selective pressure have led to an increase in the number of organisms that are resistant to the effects of antibiotics[5]. The increase in antibiotic resistance among a wide range of bacteria has caused some people to speculate that we are in danger of seeing an end to the antibiotic era[5].

*Corresponding author: Dr. Okwori EE, Department of Medical Microbiology and Parasitology, College of Health Sciences, Benue State University.
Tel: 08035734145
E-mail: drejokwori@yahoo.com

The success of antibiotic chemotherapy may often depend on the isolation and the determination of the susceptibility pattern of the causative organism. In developing countries, this practice is rare in most health institutions due to the lack of facilities and personnel[6]. Multi-resistance of many bacteria to many antibiotics has been reported in the developing countries due to misuse of antibiotics[4,6]. A way of combating the development of resistance is to control usage and formulation of antibiotic policy. The surveillance of the susceptibility patterns of the organisms prevalent in the individual hospital and community such as ours at Otukpo General Hospital will help to control resistance. Otukpo has attracted the attention of many interest groups both internationally and nationally since the advent of upsurge of HIV in the area. Many groups of people come to the area to practice medicine. In addition, the study will help in the establishment of antibiotic policy in the area. The management can also be guided by the pattern of antibiotic susceptibility in the procurement of various antibiotics for the hospital. The physicians can also be guided in their choice of antibiotics in severe infections while waiting for the result of antibiotic sensitivity tests.

2. Materials and methods

2.1. Study center

Otukpo General Hospital was chosen for the study. Otukpo is the traditional headquarter of Idoma people. It is located in the North Central part of Nigeria, bounded by Enugu State in the east, Cross River State in the west, Makurdi in the north and Kogi State in the south. The laboratory at the General Hospital serves the whole of Idoma people and some people around the area.

The laboratory was equipped by the management of Department for International Development (DFID), a British non-governmental organization. DFID also obtained ethnical clearance from the Benue State Ministry of Health for the research wing of the project. For the purpose of the research those samples processed in the laboratory during that three years of the research were processed free of charge for the patients.

The different samples coming into the laboratory at that research period (1997 to 2000) were used for the work. 20000 samples were received from patients attending clinics and in patients of the hospital and analyzed.

2.2. Bacteria isolation and identification

18520 isolates were obtained from the 20000 samples. Morphological appearance of the colonies, biochemical tests and serology were used to identify the organisms. Enterobacteriaceae were identified as follows using standard biochemical reaction (Table 1).

Table 1
Biochemical tests used to identify the Enterobacteriaceae.

Non-lactose fermenters	Cit	Urea	H_2S	Motility	Man	Ornith
Salmonella typhi	+	−	+	+	+	
Salmonella paratyphi A	+	−	+	+	+	
Salmonella paratyphi B	+	−	+	+	+	
Salmonella paratyphi C	+	−	+	+	+	
Shigella species	−	−	−	−	−	
Shigella dysenteriae	−	−	−	−	−	
Shigella flexneri	−	−	−	−	+	
Shigella boydii	−	−	−	−	+	
Shigella sonnei	−	−	−	−	+	+
Proteus sp.	+	+	+	+	−	+
Pseudomonas sp.	Oxidase positive					

Final identification of *Salmonella* was based on their serological reaction. Species identification was carried out serologically. There were commercial kits used to agglutinate serogroup salmonellae by their O antigen A, B, C, C_2, D and E.

2.3. Susceptibility testing

The tests were performed on diagnostic sensitivity test-Muller-Hinton agar, supplemented with 5% sheep blood and chocolate agar. The inoculum of both test and control organisms were prepared from overnight growth and adjusted to a turbidity equivalent to 0.5 and 1 McFarland opacity standard. The procedures were carried out according to standard recommendations[7]. Antibiotic susceptibility tests were done against locally available antibiotics using disk diffusion method in accordance with the CLSI criteria (CLSI, 2006) and similarly interpreted[8]. The following quality control organisms were used: *Escherichia coli* (*E. coli*) ATCC 25922, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC, *Streptococcus pneumonia* (*S. pneumonia*) ATCC 49619. After the agar surface was dry, the following antimicrobial discs including ampicillin (10 mg), cotrimoxazole (25 mg), clindamycin (2 mg), chloramphenicol (30 mg), ceftriaxone (30 mg), penicillin (2 mg), flucloxacillin (5 mg), erythromycin (15 mg), ciprofloxacin (5 mg), nitrofurantoin (10 mg), amoxicillin/clavulinic acid (augmentin) (20/10 mg), were applied and the plates were incubated both aerobically and anaerobically at 37 ℃ for 24 to 48 hours.

2.4. Statistical analysis

The results were analysed using statistical package for the social sciences (SPSS) 11.0 statistical softwares. *Chi*-square χ^2 was used to compare associations between proportions and *P*-values <0.05 were considered significant at 95% confidence limit.

3. Results

The specimens used from nine different clinical sites were as follows: wounds 4230 (22.84%), urine 5865 (31.67%), blood 2292 (12.38%), genital 1426 (7.70%), sputum 1262 (6.81%), stool 1163 (6.28%), cerebrospinal fluid (CSF) 1108 (5.98%), aspirates 713 (3.85%) and ear/throat 461 (2.49%).

Staphylococcus species was the commonest gram positive bacteria isolated from all the specimens. It accounted for 2511 (13.56%) of the isolates, followed by *S. pneumonia* which was 760 (4.10%) of the total isolates. *Pseudomonas* specimen was the most predominant, 2238 (12.08%) of the gram negative bacteria followed by *E. coli* 2073 (11.19%). *Neisseria gonorrheae* (*N. gonorrheae*) formed 1119 (6.04%) of the total isolates, and they were from urine and genital. *Salmonella* accounted for 1707 (9.22%) of the isolates. 548 (2.96%) of the isolates were *Neisseria meningitidis* (*N. meningitidis*) and all came from CSF (Table 2).

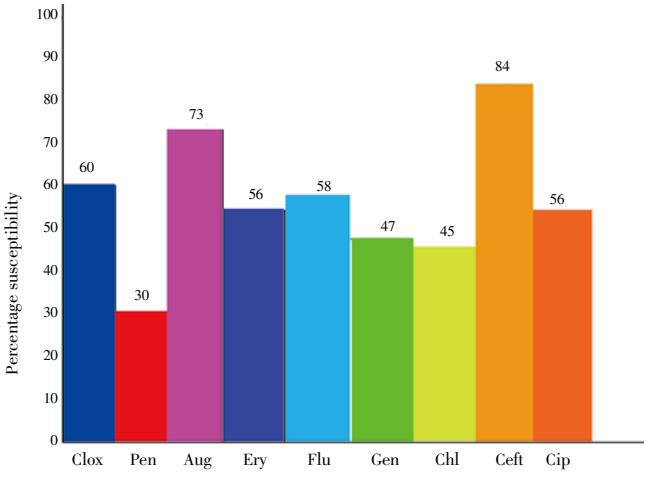

Figure 1. Percentage susceptibility of *S. aureus* to some antibiotics. Clox: cloxacillin; Pen: penicillin; Aug: augmentin; Ery: erythromycin; Flu: flucoxacillin; Gen: genticin; Chl: chloramphenicol; Ceft: ceftriaxone; Cip: ciproxin.

Table 2
Distribution of major groups of bacteria isolated from the specimens [*n* (%)] (*n*=18 520).

	Bacteria	Wound	Urine	Genital	Blood	Sputum	CSF	Stool	Ear & throat	Aspirates	Total
Gram positive	*S. aureus*	770	469	353	469	120	60	–	80	190	2 511 (13.56)
	S. pyogenes	50	0	30	15	130	23	0	50	30	328 (1.77)
	S. pneumonia	0	0	0	50	380	250	0	80	0	760 (4.10)
	Streptococcus faecalis	18	250	5	0	0	50	50	30	39	442 (2.39)
	Streptococcus viridans	120	20	5	15	150	60	0	30	5	405 (2.19)
Gram negative	*Pseudomonas*	1 200	600	5	90	40	0	33	20	250	2 238 (12.08)
	E. coli	520	1 200	10	90	10	60	50	78	55	2 073 (11.19)
	Proteus sp.	420	500	30	0	0	0	0	0	0	950 (5.13)
	Providencia	250	950	15	0	0	0	0	19	25	1 259 (6.80)
	Klebsiella	300	1 028	23	0	520	0	0	0	0	1 871 (10.10)
	N. gonorrheae	0	139	950	30	0	0	0	0	0	1 119 (6.04)
	S. typhi	190	390	0	590	10	59	380	53	33	1 707 (9.22)
	Salmonella paratyphi A	120	180	0	340	0	13	280	13	39	985 (5.32)
	Salmonella paratyphi B	90	100	0	280	0	5	190	5	19	689 (3.72)
	Salmonella paratyphi C	180	39	0	180	17	8	180	3	28	635 (3.43)
	N. meningitidis	0	0	0	28	0	520	0	0	0	548 (2.96)
	Total	4 230 (22.84)	5 865 (31.67)	1 426 (7.7)	2 292 (12.38)	12 625 (6.81)	1 108 (5.96)	1 163 (6.28)	461 (2.49)	713 (3.85)	18 520

Table 3
Antibiotic sensitivity pattern of the bacteria (%).

Bacteria	Pen	Amp	Clox	Aug	Chl	Ery	Cot	Gen	Nit	Flu	Ceft	Clin	Cip
S. aureus	30	35	60	73	45	56	37	47	–		84	43	56
S. pyogenes	84	74	63	94	41	34	46	58	13	43	78	41	73
S. pneumonia	93	71	63	89	33	37	38	75	28	58	81	40	69
Streptococcus viridans	58	63	59	81	43	53	38	60	15	45	81	44	70
E. coli	0	53	47	81	63	41	54	58	60	63	91	53	75
Proteus sp.	0	53	47	81	63	41	54	58	60	63	91	53	96
Providencia	3	34	41	65	45	3	48	53	50	54	87	51	73
Klebsiella	0	12	17	54	38	12	25	48	17	39	68	48	65
Pseudomonas	0	0	0	20	0	0	0	39	0	5	53	0	58
N. gonorrheae	10	6	3	20	0	0	5	38	0	17	63	58	73
S. typhi	0	31	5	48	51	0	28	51	0	18	75	17	81
Salmonella paratyphi A	0	28	17	53	50	0	41	58	0	15	81	0	83
Salmonella paratyphi B	0	38	20	61	53	0	50	73	0	28	85	0	90
Salmonella paratyphi C	0	40	30	71	51	0	48	68	20	31	78	14	81
N. meningitidis	63	58	43	71	53	48	41	63	15	28	74	60	63

Pen: penicillin; Amp: Ampicillin; Clox: cloxacillin; Aug: Augmentin; Chl: chloramphenicol; Ery: erythromycin; Cot: cotrimoxazole; Gen: genticin; Nit: nitrofurantoin; Flu: floxacillin; Ceft: ceftriaxone; Clin: clindamycin; Cip: ciproxin.

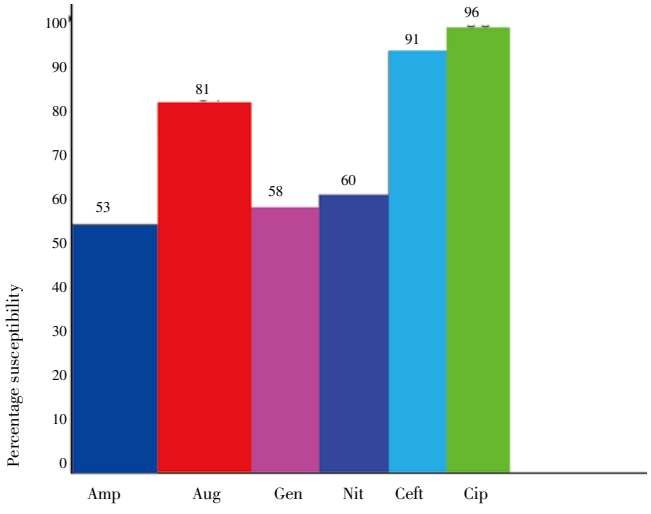

Figure 2. Percentage susceptibility of *E. coli* to some antibiotics.
Amp: ampicillin; Aug: augmentin; Gen: Genticin; Nit: nitrofurantoin; Ceft: ceftriazone; Cip: ciproxin.

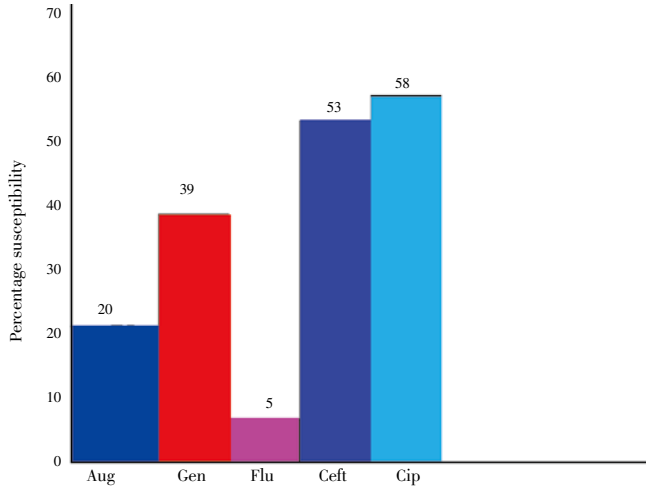

Figure 3. Percentage susceptibility of *Pseudomonas* to some antibiotics.
Aug: augmentin; Gen: gentin; Flu: flucoxacillin; Ceft: ceftriaxone; Cip: ciproxin.

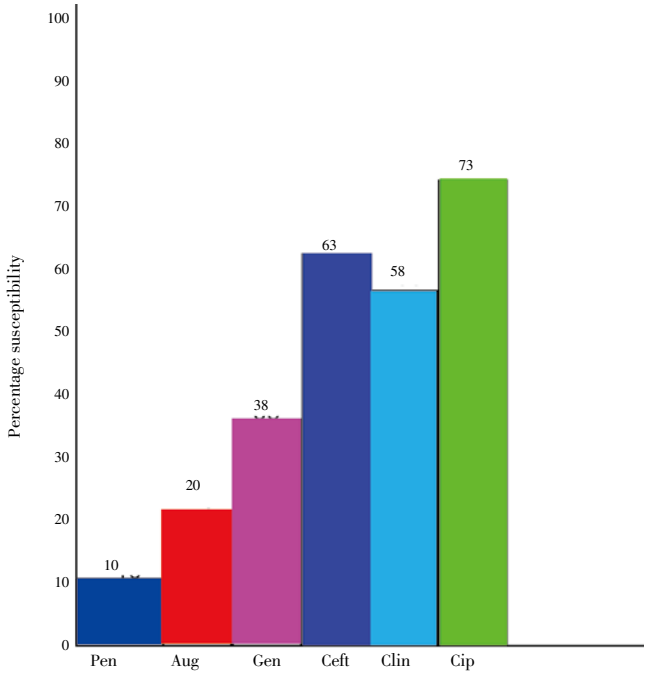

Figure 4. Percentage susceptibility of *N. gonorhoeae* to some antibiotics.
Pen: penicillin; Aug: augmentin; Gen: genticin; Ceft: ceftriaxone; Clin: clindamycin; Cip: ciproxin.

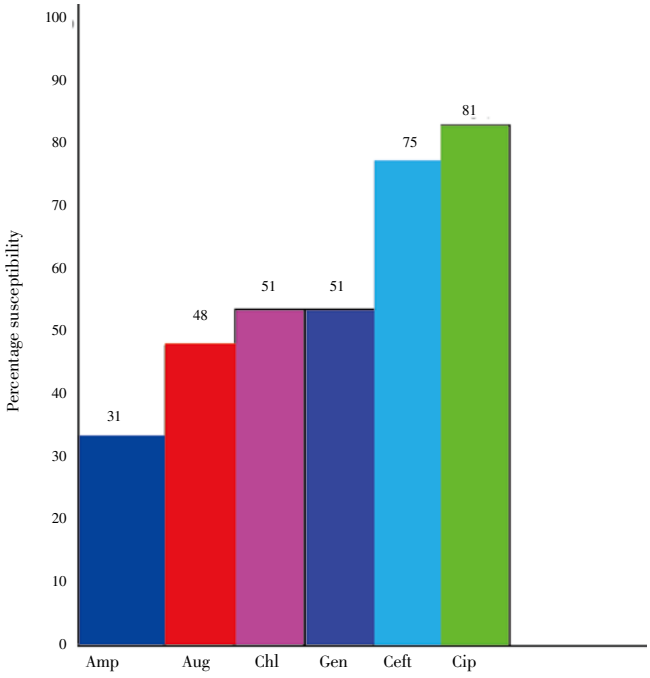

Figure 5. Percentage susceptibility of *S. typhi* to some antibiotics.
Amp: ampicillin; Aug: augmentin; Chl: chloramphenicol; Gen: genticin; Ceft: ceftriaxone; Cip: ciproxin.

770 (4.16%) isolated from the wound infection were *Staphylococcus aureus* (*S. aureus*) while *Pseudomonas* accounted for 1 200 (6.48%) of the total isolates from the wound. The commonest bacteria isolated from CSF was *N. meningitidis* which was 520 (2.81%), followed by *S. pneumonia* 250 (1.35%). 950 (5.13%) of the genital isolates were *N. gonorrheae* (Table 2).

Table 3 showed that 30% of *S. aureus* was sensitive to penicillin, while 73% and 84% were sensitive to augmentin and ceftriaxone, respectively and showed 56% sensitivity to erythromycin (Figure 1).

Streptococcus pyogenes (*S. pyogenes*) showed 84%, 74%, 94% sensitive to penicillin, ampicillin and augmentin, respectively and 78% and 73% sensitive to ceftriaxone and ciproxin, respectively.

E. coli showed 53% and 81% sensitivity to chlorampenicol and augmentin, respectively (Figure 2). Only 58% of them were sensitive to gentamicin. But 91% and 96% of them were sensitive to ceftriaxone and ciproxin, respectively. 20%, 39% isolates of the *P. aeruginosa* were sensitive to augmentin and gentamicin, respectively (Figure 3). 53% and 58% of them were sensitive to ceftriaxone and ciproxin (Table 3).

N. gonorrheae showed 63% and 73% sensitivity to ceftriaxone and ciproxin, respectively (Figure 4). *Salmonella typhi* (*S. typhi*) showed 48% sensitivity to augmentin and 75% and 81% of them were sensitive to ceftriaxone and ciproxin, respectively (Figure 5).

37% of the *N. meningitidis* were resistant to penicillin, but showed 58% and 71% sensitivity to ampicillin and augmentin, respectively, 74% of them were sensitive to ceftriaxone (Figure 6).

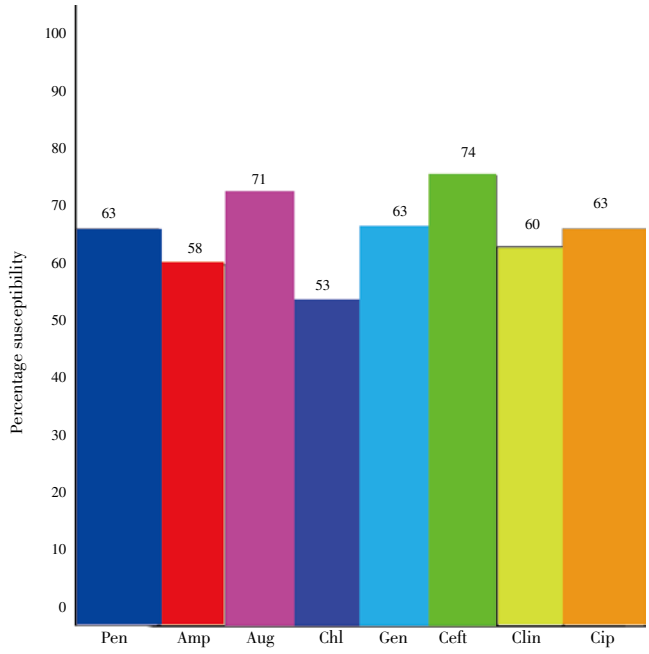

Figure 6. Percentage susceptibility of *N. meningitidis* to some antibiotics.
Pen: penicillin; Amp: ampencillin; Aug: augmentin; Chl: chloramphenicol; Gen: genticin; Ceft: ceftriaxone; Clin: clindamycin; Cip: ciproxin.

4. Discussion

Majority of the bacteria isolated from the specimens in this study were gram negatives 14 074 (76%). *Pseudomonas* and *E. coli* were the most predominant of the gram negative organisms. This may be because *E. coli* form part of normal gut flora and *Pseudomonas* is abundant on the body surfaces and materials. They are therefore expected to be more likely involved in urinary tract and wound infections. Some of the infections may be auto-infection, especially in females.

Pseudomonas was the highest isolated from wound infection 1 200 (6.48%) followed by *S. aureus* 770 (4.16%). These two organisms are abundant on human surface, and natural materials so are more frequently encountered as causes of wound infection or contamination[5]. They are observed to be resistant to the most commonly used antibiotic in the

area, *i.e.* penicillin (70%), ampicillin (65%) and cotrimoxazole (63%). *Pseudomonas* and *E. coli* which were the highest encountered gram negative bacteria in this work showed a lot of resistance to the common antibiotic in the community.

For example, *E. coli* showed 47%, 42% and 46% resistance to ampicillin, gentamicin and cotrimoxazole, respectively. This may be as a result of abuse of the usage of the antibiotics in the area. These drugs are frequently sold by vendors in the community[6,7]. *Pseudomonas* showed sensitivity to augmentin 20%, genticin 39%, ceftrixone 53% and ciproxin 58%.

It is a welcome development that we now have good number of antibiotics that are working against *Pseudomonas*.

N. gonorrheae showed 62% resistance to genticin and surprisingly 47% and 27% resistance to ceftriaxone and ciproxin, respectively. The abuse of the drugs in the area is on the increase and many organisms are now showing resistance to them in reasonable quantity.

49% of *S. typhi* are resistant to chloraphenicol and genticin while 25% and 19% are resistant to cefrtriaxone and ciproxin, respectively.

It is a very dangerous trend as it will soon become very difficult to treat typhoid and paratyphoid fevers in the area.

The question is where do we go from here if resistance has now spread to the new generation cephalosporins and ciproxin? If no effort is done to control the use of antibiotic in the community, then we are heading for doom in terms of antibiotic usage. The clinical microbiologist would have an important role in providing his clinical colleagues with the latest surveys of the prevalence of pathogenic bacteria and their susceptibility pattern[7,8]. Such surveillance information can also be used in the formulation policies for the supply and use of antibiotics in the community.

Studies from central Europe show that bacterial resistance to antimicrobial agents has not changed much during the last 10 years[9]. Also in USA, for the past 12 years, there is no much change in the resistance of microbial to antibiotics[10]. Geographical variation can affect the antibiotics resistance pattern which was also noticed in UK, so there is a need for local surveillance from time to time[9,10].

In our environment and in most places, the choice of antibiotics is often made and treatment started before laboratory report of the susceptibility of the causative organism is available. So there is high need for clinicians to be aware of local infecting organisms and their antibiotic susceptibility and resistance patterns in any given community. This will guide them in a best principle to be applied in the treatment of their patients. It is always important that clinicians should obtain necessary specimens before commencing antibiotic therapy.

This knowledge is only possible if we have functional laboratories with trained personnel in our hospitals. It will then be essential to base prescribing policy on local statistics, in view of the fact that geographical variation exists as causes of infection and their susceptibility to antibiotics.

It is also important, that laboratories should have information on the prescribing policies of the clinicians they serve.

Earlier studies have shown that education and training strategies aimed at doctors have shown to be very useful in the proper use of antimicrobials[10,11]. For this success to continue, adequate surveillance on the resistance of bacteria to antimicrobials agents must be maintained.

We are of the view that by instigating a national survey of bacterial resistance increased awareness of their problems will hopefully assist to reduce the incidence of hospital acquired infection and antibiotic resistance. In the USA, this has been reported as a major advantage of surveillance[11,12]. The present study was intended to bring to the notice of health officials in the area the importance of surveillance on how data collected could be used effectively to improve management of various bacteria in Otukpo area.

Conflict of interest statement

We declare that we have no conflict of interest.

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