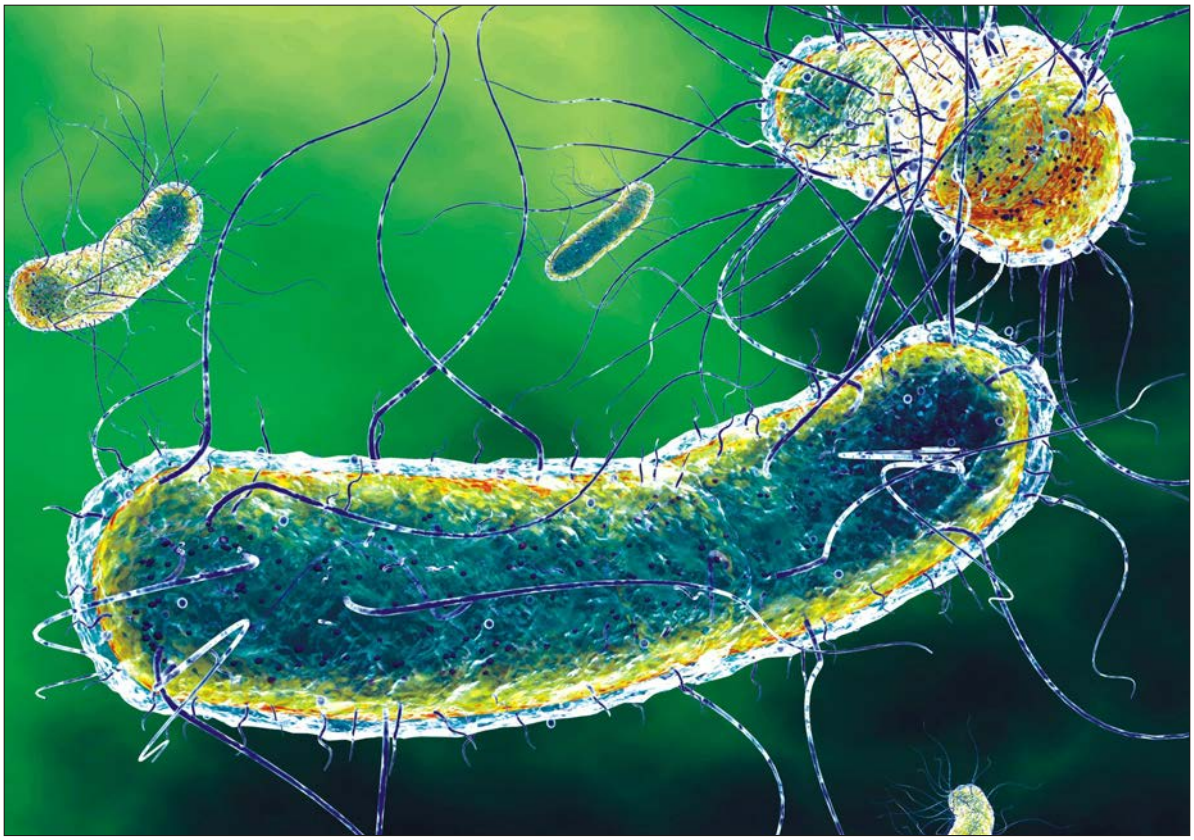

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Antibiogram Study of *Proteus* spp. Bacterial Isolates from Uropathogenic Infections in University of Benin Teaching Hospital, Nigeria

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ABSTRACT

This study investigates the prevalence of *Proteus* spp. bacterial isolates from uropathogenic infections as well as their susceptibility and antibiogram patterns to some selected antibiotics. A total of 1927 patients presenting at University of Benin Teaching Hospital were screened for Urinary Tract Infection (UTI). Of the total 1927 sample studied, 14.58% were positive for UTI. About 281 samples positive for UTI, 14.59% were positive for *Proteus* spp. distributed in to *Proteus mirabilis* (68.29%) and *Proteus morganii* (31.71%). The highest distribution was observed in the month of September, followed by August and November with July, October and December presenting the least. *Proteus mirabilis* was not susceptible to Cloxacilin, Erythromycin and Cephalexine but was highly sensitive to Peflacine, Ciproxin, Cefuroxine and Ofloxacin. *Proteus morganii* was not susceptible to Ampiclin, Amoxicillin, Ofloxacin, Ciproxin, Cephalexin and Cefotaxime but was highly sensitive to Cloxacillin, Genticin and Cefuroxime. The Ajumali's mnemonic coding showed that no two strains of any of *Proteus mirabilis* and *Proteus morganii* were same. Judging by these results, multidrug-resistant *Proteus mirabilis* and *Proteus morganii* is becoming a growing public health problem and hence, the need for antimicrobial sensitivity screening for accurate antibiotic prescription is becoming high.

Key words: Urinary tract infection, *Proteus mirabilis, Proteus morganii,* antibiotic, public health problem

INTRODUCTION

Urinary Tract Infection (UTI) is one of the most common bacterial infection in humans both in the community and hospital settings and are the most common bacterial infections encountered by clinicians in developing countries (Dalela *et al.*, 2012; Kashef *et al.*, 2010). Worldwide, approximately 150 million people are diagnosed with UTIs resulting in over USD 6 billion health care expenditures (Weichhart *et al.*, 2008).

Worrisome is the fact that drug resistance among bacteria causing UTI has increased since the introduction to UTI chemotherapy (Nerurkar *et al.*, 2012; Sood and Gupta, 2012; Bahadin *et al.*, 2011; Haider *et al.*, 2010; Tseng *et al.*, 2008). Besides, the etiological agents and their susceptibility patterns of UTI vary in regions and geographical locations. According to De Francesco *et al.* (2007), the etiologyand drug resistance change through time. Hence, knowledge of the local bacterial etiology and susceptibility patterns is required to trace any change that might have occurred in time so that updated recommendation for optimal empirical therapy of UTI can be made as reported by Leegaard *et al.* (2000).

In this study, the *Proteus* species are of interest which according to De Champs *et al.* (2000) are a major cause of diseases acquired outside the hospital and in cases eventually require hospitalization. *Proteus* is a genus of gram-negative bacteria belonging to the family of Enterobactericeae. They are distinguishable from other genera by their ability to swarm across an agar surface (Jacobsen *et al.*, 2008). *Proteus* ranks third as the cause of hospital-acquired infections (Stamm, 1999) and they are widespread in the environment and makes up part of the normal flora of the human gastrointestinal tract.

Significantly, there has been a report in Europe on the evolution and spread of multidrug-resistant *Proteus mirabilis* clone with chromosomal AmpC-type beta-lactamase (D'Andrea *et al.*, 2011; Luzzaro *et al.*, 2009). In addition, a report exists on a wide diversity between institutions in the prevalence of pathogens and in their antimicrobial susceptibility (Fridkin, 2001) and it is said to be particularly worse in resource-poor countries where sale of antibiotics is poorly controlled (Onile, 1997).

Considering the literatures above, spread of multidrug-resistant pathogen is representing a growing public health problem in the world. In fact, with the increasing phenomenal evolution and multidrug-resistance of many bacterial pathogens (with special interest on multidrug-resistance *Proteus* spp.), there is the need for regular review of antimicrobial sensitivity pattern among clinically isolated *Proteus* spp. for accurate decision on antibiotic prescription. This study was therefore, designed to investigate the prevalence of *Proteus* spp. bacterial isolates from uropathogenic infections as well as their susceptibility and antibiogram patterns to some selected antibiotics.

MATERIALS AND METHODS

Specimen: A total of 1,927 clinical specimen comprising of Mid-Stream Urine (MSU), Super-Public Urine (SPU) and catheter specimen were collected from in-and-out patients in UBTH, between July, 2009 and December, 2009 for this study (Orhue, 2004). These samples were taken to the laboratory for standard microbiological analysis with 30 min of collection.

Isolation and identification: The specimen was inoculated onto nutrient agar, blood agar and MacConkey agar plates by streaking. Inoculated plates were then incubated aerobically at 37°C for 24 h. After 24 h of incubation, discrete colonies were picked up and gram stained and further subculturing was done to obtain pure cultures and biochemical tests carried out.

Antibiotics under study are as follows (Momoh *et al.*, 2011):

- **Ciprofloxacin:** This drug is a fluoroquinolone and acts by inhibiting DNA topoisomerases (gyrases); thereby, inhibiting bacterial DNA synthesis
- **Erythromycin:** This drug belongs to the class of macrolide, it is bacteriostatic, binding to the 23-RNA of the 50s ribosomal subunit to inhibit peptide chain elongation during protein synthesis
- **Augmentin:** This is a combination of amoxycillin and clavulinc acid. The clavulinic acid helps protect the amoxycillin from being inactivated by the enzyme beta-lactamsae, an enzyme produced by pathogenic bacteria
- **Gentamycin:** This drug is an aminoglycoside which binds to small ribosome subunits and interfere with protein synthesis by directly inhibiting protein synthesis
- **Cefuroxime:** This is a broad spectum antibiotic of the cephalosporin class. It is an alternate drug of choice when patients are allergic to the penicillins or when there is a need to overcome beta-lactamase inactivation

Antibiotics susceptibility testing (antibiogram): This was done by the multi-discs diffusion using 21 different antibiotics. The multi discs were placed on the plates which were previously inoculated, few minutes earlier, then the plates were incubated at 37°C for 24 h, thereafter, the plates were examined for zones of inhibition around the different antibiotic disc. *Staphylococcus aureus* Oxford stain NTC 6751 was used as control for gram positive organisms.

Mnemonic coding: The Ajumali's mnemonic coding method as earlier described by Joghi *et al.* (1984), was adopted as a typing scheme to re-arrange the nominal antibiotics into arbitrary numeric values, making it easy for the differentiation of strains. Using this pneumonic coding scheme, a sensitive result was scored as (+) while a resistance was scored as (-). Also, the 21 different antibiotics were divided into a group of 3 antibiotics each, following their mechanisms of action as well as, their clinical applications and these 3 antibiotics were given numerical values of 1, 2 and 4.

Thus, a perfect sensitivity to the 3 antibiotics will give a summation of 1+2+4 = 7. While complete resistance to the 3 antibiotics will give a summation of 0+0+0 = 0. The other values as obtained by adding up these numerical values thus, an isolate can receive a score of 0-7 in each triplet segment which, when the seven triplet segments are combined together, gives a seven (7) digit numerical value as the antibiogram types (Orhue, 2004).

Data analysis: All data was analyzed using simple descriptive statistic.

RESULTS

Of the total 1927 sample, 14.58% (281 samples) were positive for UTI among whom female represents 15.37% and male 13.46% (Table 1). Among the 281 samples positive for UTI, 14.59% were positive for *Proteus* spp. *Proteus mirabilis* (68.29%) and *Proteus morganii* (31.71%) were the *Proteus* organisms that were the only *Proteus* spp. presented (Table 2).

Table 3 shows the distribution of the *Proteus* spp. isolated during the studied period. Although, the total number in each month did not differ, however, the month of September presented the highest isolates (9 isolates) followed by the months of August and November (7 isolates each) with July, October and December producing the least isolates (6 isolates each).

Table 1: Distribution of the sampled population and prevalence of UTI

Sex	No. of samples examined	No. of positive cultures	Percentage
Male	795	107	13.46
Female	1132	174	15.37
Total	1927	281	14.58

Table 2: Prevalence of *Proteus* spp. isolated from the sampled population

	Value	
Item	No.	%
No. of positive UTI	281	
Proteus spp.	41	
Prevalence *Proteus* spp. infection	14.59	
***Proteus* spp. isolated**		
Proteus mirabilis	28	68.29
Proteus morganii	13	31.71

Table 3: Distribution of the *Proteus* spp. isolated from the sampled population

Bacterial isolates	July	August	Sept	Oct	Nov	Dec	Total
Proteus mirabilis	4	5	6	4	5	4	28
Proteus morganii	2	2	3	2	2	2	13
Total	6	7	9	6	7	6	41

Table 4: Cumulative frequency of susceptibility of bacterial isolates to antibiotics

Antibiotics	Microgram per disc	*Proteus mirabilis* (n = 28)	*Proteus morganii* (n = 13)
Ampicllin	10	14.3	0
Amoxicillin	10	21.4	0
Cloxacillin	10	0	91
Flucloxacillin	10	17.9	18.7
Amoxy clav	10	25	63.6
AmpiSulbactam	10	14.3	36.4
Genticin	10	53.6	90.9
Tobramycin	10	53.6	45.5
Streptomicin	10	32.1	18.2
Chloramphenicol	10	35.7	12.7
Tetracycline	10	17.9	36.4
Co-trimoxazole	5	42.9	45.5
Metronidazole	10	32.1	9
Nitrofurantoin	100	53.6	45.5
Erythromycin	10	0	18.2
Ofloxacin	10	64.3	0
Ciproxin	10	85.7	0
Peflacine	10	100	27.3
Cephalexine	10	0	0
Cefotaxime	30	57.1	0
Cefuroxime	10	71.46	80.6

Table 4 shows the susceptibility of the *Proteus* spp. isolates to 21 different antibiotics. *Proteus mirabilis* was not susceptible to Cloxacilin, Erythromycin and Cephalexine. On the other hand, *Proteus morganii* was not susceptible to Ampicllin, Amoxicillin, Ofloxacin, Ciproxin, Cephalexin and Cefotaxime. Higher susceptibility was observed with Peflacine, Ciproxin, Cefuroxine and Ofloxacin for the case of *Proteus mirabilis* while for the case of *Proteus morganii* high susceptibility was observed with Cloxacillin, Genticin and Cefuroxime (Table 4). Table 5 and 6 show the sensitivity and antibiogram types of *Proteus mirabilis* and *Proteus morganii*, respectively. The Ajumali's mnemonic coding showed that no two strains of any of the *Proteus* spp. were the same for the *Proteus mirabilis* and *Proteus morganii*.

DISCUSSION

In the current study, the prevalence of UTI observed to be 14.58% with *Proteus* spp. infection accounting for 14.59% of cases. Although, two strains of *Proteus* spp. were observed, the most prevalence was *Proteus mirabilis* accounting for 68.29% and *Proteus morganii* accounting for 31.71%. This high prevalence of *Proteus* spp. in UTI is not in line to fact that *Proteus* is seems to be a common cause of wound infections in West Africa (Feglo *et al*., 2010; Newman *et al*., 2006; Yah *et al*., 2007) but agrees with the report in Europe and Asia that *Proteus* is commonly encountered in urine than in other clinical specimens (Reslinski *et al*., 2005; Orrett, 1999).

According to the finding of this study, *Proteus* spp. infection was highest in the month of September. This finding is similar to the study by Bahashwan and El Shafey (2013)

Table 5: Sensitivity and antibiogram of *Proteus mirabilis*

P. mirabihis isolates Key	Ampicillin 1	Amoxicillin 2	Cloxacillin 4	Flucloxacillin 1	Amoxy clav 2	Ampisulbactam 4	Genticin 1	Tobramycin 2	Streptomycin 4	Chloramphenicol 1	Tetracycline 2	Antibiogram type
1	+	-	-	-	-	-	+	+	+	+	-	1075676
2	-	-	-	-	-	-	+	-	-	+	-	0015066
3	-	-	-	-	+	-	+	+	+	-	-	270376
4	-	-	-	-	-	-	-	-	-	-	-	0000274
5	-	+	-	+	-	-	+	+	+	-	-	2174356
6	-	-	-	+	+	+	-	+	-	+	-	0701376
7	-	+	-	-	+	-	-	+	-	-	-	2220070
8	-	-	-	-	-	-	+	+	+	+	+	0077276
9	+	-	-	+	+	-	-	+	+	-	+	0662376
10	-	-	+	+	-	+	-	+	-	-	-	4500276
11	-	-	-	-	-	-	+	+	-	-	-	0030244
12	-	-	-	-	-	-	-	+	+	+	+	0063242
13	-	-	-	-	-	-	-	+	-	+	+	0023074
14	-	-	-	+	-	-	+	+	+	-	-	0064142
15	-	+		+	+	-	+	-	+	-	-	2354276
16	-	-	-	-	-	-	-	-	-	-	-	0000074
17	+	+	-	+	+	-	+	-	-	-	+	3312076
18	-	+	-	-	-	-	-	+	+	-	-	2060376
19	-	-	-	-	-	-	+	+	-	-	-	0000160
20	-	-	-	-	-	-	+	+	-	+	-	0035262
21	-	-	-	-	-	-	-	+	-	+	-	0025272
22	+	-	-	-	-	-	-	-	-	-	+	1045232
23	-	-	-	-	-	-	-	-	-	-	-	0006074
24	-	-	-	-	-	-	+	-	-	+	+	0000272
25	-	+	-	-	+	-	+	+	-	-	-	2234264
26	-	-	-	+	+	+	-	-	-	-	-	0700074
27	-	-	-	-	-	-	+	+	-	+	+	0037176
28	-	-	-	-	-	-	-	-	-	-	+	0002276

Table 5: Continue

P. mirabihis isolates Key	Co-trimoxazole 4	Metronidazole 1	Nitrofurantoin 2	Erythromycin 4	Ofloxacin 1	Ciproxin 2	Peflacine 4	Cephalexin 1	Cefotaxime 2	Cefuroxime 4	Antibiogram type
1	+	-	+	-	+	+	+	-	+	+	1075676
2	+	-	-	-	+	+	+	-	+	+	0015066
3	-	+	+	-	+	+	+	-	-	+	0270376
4	-	-	+	-	+	-	+	-	+	+	0000274
5	+	+	+	-	+	+	+	-	+	+	2174356
6	-	-	+	-	+	+	+	-	-	-	0701376
7	-	-	-	-	+	+	+	-	-	-	2220070
8	+	-	+	-	+	+	+	-	+	+	0077276
9	-	+	+	-	+	+	+	-	+	+	0662376
10	-	-	+	-	-	-	+	-	-	+	4500276
11	-	-	+	-	-	-	+	-	+	-	0030244
12	-	-	+	-	-	-	+	-	+	-	0063242
13	-	-	-	-	+	+	+	-	-	+	0023074
14	+	+	-	-	-	-	+	-	+	-	0064142
15	+	-	+	-	+	+	+	-	+	+	2354276
16	-	-	-	-	+	+	+	-	-	+	0000074
17	-	-	-	-	+	+	+	-	+	+	3312076
18	-	+	+	-	+	+	+	-	+	+	2060376
19	+	+	-	-	-	+	+	-	-	-	0000160
20	+	-	+	-	-	+	+	-	+	-	0035262
21	+	-	+	-	+	+	+	-	+	-	0025272
22	+	-	-	-	-	+	+	-	+	-	1045232
23	-	-	+	-	+	+	+	-	-	+	0006074
24	-	-	-	-	+	+	+	-	+	+	0000272
25	+	-	+	-	-	+	+	-	-	+	2234264
26	-	+	-	-	+	+	+	-	-	+	0700074
27	+	+	-	-	+	+	+	-	+	+	0037176
28	-	-	+	-	+	+	+	-	+	+	0002276

Table 6: Sensitivity and antibiogram types of *Proteus morganii*

P. mirabihis isolates	Ampicillin	Amoxicillin	Cloxacillin	Flucloxacillin	Amoxyclav	Ampisulbactam	Genticin	Tobramycin	Streptomycin	Chloramphenicol	Tetracycline	Antibiogram
Key	1	2	4	1	2	4	1	2	4	1	2	type
1	-	-	-	-	+	+	-	-	-	+	+	0603076
2	-	+	-	-	+	+	+	+	-	+	-	2605076
3	-	-	-	-	+	+	-	+	+	+	+	0207276
4	-	-	-	-	+	-	+	+	+	+	+	3667262
5	+	+	-	+	+	+	+	-	-	-	+	3716236
6	-	-	-	+	-	-	-	+	+	+	-	0161156
7	-	-	-	-	+	+	+	+	-	+	-	0635144
8	-	-	-	-	+	+	-	-	-	-	-	0104076
9	-	+	-	-	-	-	-	+	-	-	-	2024354
10	-	-	-	-	-	+	+	-	+	+	-	0411352
11	-	-	-	-	+	-	-	+	+	+	+	0263272
12	+	-	-	-	+	-	-	+	-	+	-	1201176
13	-	-	-	-	-	+	+	+	+	+	+	0473066

P. mirabihis isolates	Co-trimoxazole	Metronidazole	Nitrofurantoin	Erythromycin	Ofloxacin	Ciproxin	Peflacine	Cephalexin	Cefotaxime	Cefuroxime	Antibiogram
Key	4	1	2	4	1	2	4	1	2	4	type
1	-	-	-	-	+	+	+	-	+	+	0603076
2	+	-	-	-	+	+	+	-	+	+	2605076
3	+	-	+	-	+	+	+	-	+	+	0207276
4	+	-	+	-	-	+	+	-	+	-	3667262
5	+	-	+	-	+	+	-	-	+	+	3716236
6	-	+	-	-	+	-	+	-	+	+	0161156
7	+	+	-	-	-	-	+	-	-	+	0635144
8	+	-	+	-	+	+	+	-	+	+	0104076
9	+	+	+	-	+	-	+	-	-	+	2024354
10	-	+	+	-	+	-	+	-	+	-	0411352
11	-	-	+	-	+	+	+	-	+	-	0263272
12	-	+	-	-	+	+	+	-	+	+	1201176
13	-	-	-	-	-	+	+	-	+	+	0473066

who reported *Proteus* spp. infections to be highest during summer season from 22 June to 22 September. Indeed, studies have reported relationship between bacterial infections and seasonal variation (Bryan, 2011; Smith and Hogan, 2008; O'Hara *et al.*, 2000) and it has long been known that bacterial infections peak during summer season.

Interestingly, *Proteus* spp. acted differently in terms of sensitivity and resistivity to the studied antibiotics and similar report has been documented by Bahashwan and El Shafey (2013). In this study, the isolated *Proteus* spp. showed diversity in antibiotic susceptibility and sensitivity. In fact, the most effective antibiotics for *Proteus mirabilis* were Peflacine, Ciproxin, Cefuroxine and Ofloxacin while for *Proteus morganii* were Cloxacillin, Genticin and Cefuroxime. Specifically, *Proteus mirabilis* was resistance to Cloxacilin, Erythromycin and Cephalexine while *Proteus morganii* was resistance to Ampicllin, Amoxicillin, Ofloxacin, Ciproxin, Cephalexin and Cefotaxime. In line with the finding of this study, resistance of *Proteus* spp. against ampicillin, Doxycycline, Amoxycillin, Cephalothin, Erythromycin and some other antibiotics have been reported (Kibret and Abera, 2014; Feglo *et al.*, 2010; Newman *et al.*, 2006; Dance *et al.*, 1987; Chow *et al.*, 1979). Increasing drug resistance to these and other antimicrobials has been documented from previous studies (Tseng *et al.*, 2008), hence, increase in immergence of antimicrobial drug resistance. This study is therefore, a step towards the generation of national data on the prevalence of antimicrobial resistance patterns of *Proteus* spp.

The Ajumali's coding of *Proteus mirabilis* and *Proteus morganii* (Table 5 and 6) presented different antibiogram type, making them phenotypically different from one another, even though they are of the same species. This indicates a higher resolving strain differentiation effect of the *Proteus mirabilis* and *Proteus morganii*. This typing method is so specific that it can easily pass off as a phenotypic DNA antibiogram typing method (Momoh *et al.*, 2011). This therefore, indicates that an appropriate pneumonic coding can be able to resolve strains of the same microorganisms into their different and specific antibiogram types. By implication this information is very important for laboratory physician as those with knowledge of the various strain distribution and differentiation can tackle Multi-Drug Resistant (MDR) strains effectively (Momoh *et al.*, 2011).

CONCLUSION

This study has demonstrated that there is high prevalence of *Proteus mirabilis* and *Proteus morganii* in UTI in the community under study and these strains of *Proteus* spp. are with higher antimicrobial resistance. Hence, multidrug-resistant *Proteus mirabilis* and *Proteus morganii* is becoming a growing public health problem. It is therefore, recommended that there is need to fund research to regularly review antimicrobial sensitivity pattern as this will be beneficial for clinician and laboratory physician when selecting antibiotic for prescription. In addition, the need for Antibiotics susceptibility testing for patients before prescribing antibiotics cannot be overemphasized as this may reduce the growing multidrug-resistance.

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