Occurrence, species distribution and antibiotic resistance of *Proteus* isolates: A case study at the Komfo Anokye Teaching Hospital (KATH) in Ghana

Patrick Kwame Feglo[1], Stephen Yao Gbedema[2*], Solomon Nii Armar Quay[1], Yaw Adu-Sarkodie[1], Clement Opoku-Okrah[3]

[1]Department of Clinical Microbiology, School of Medical Sciences, College of Health Sciences, KNUST, Kumasi, Ghana.
[2]Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, KNUST, Kumasi, Ghana. *Corresponding author.
[3]Department of Medical Laboratory Technology, Faculty of Allied Health Sciences, College of Health Sciences, KNUST, Kumasi, Ghana.

ABSTRACT

Background and purpose: Different *Proteus* species may vary with the type of infections they cause in both the community and hospital environments. However, in many laboratories in developing countries, differentiation of the genus *Proteus* into species is not generally done during bacteriological diagnosis due to high cost and special skills involved. This study aimed at determining the prevalence of different *Proteus* species in KATH, their antibiotic resistance pattern and how they relate to patients' demographic data.
Method: This is a prospective study involving the analyses of clinical samples for *Proteus* species and determining their antimicrobial susceptibility pattern.
Results: Two hundred *Proteus* species were isolated from 2361 clinical specimens obtained from patients suspected of bacterial infection, giving 8.4 % prevalence of *Proteus* infections. Wound isolates were the highest (64.5 %) followed by ear swab. Three *Proteus* species; *P. mirabilis, P. vulgaris* and *P. penneri* were recovered from the samples. *P. mirabilis* was the commonest species (61.5 %), and hence the causative species of a majority of *Proteus* infections followed by *P. vulgaris* (30.5 %), and *P. penneri* (8 %). There was no significant difference between the out-patient and in-patient cases, just as there were no preferences for gender or age of the patients. All the species were resistant to chloramphenicol, ampicillin and co-trimoxazole. About 72.9 % of the isolates produced β-lactamase and 88.5 % were resistant to more than 2 antibiotics. *P. penneri* was the most resistant among the recovered species.
Conclusion: The three *Proteus* species recovered in KATH were all resistant to ampicillin, tetracycline, chloramphenicol and co-trimoxazole. These drugs are no longer useful in empirical treatment of *Proteus* infections at KATH. This study has also provided information for use in generating national data on the prevalence of antimicrobial resistant pathogens in Ghana.

Key words: Antimicrobial susceptibility, antibiotic resistance, *Proteus*, β-lactamase,

INTRODUCTION

Proteus species are among the commonly implicated pathogens in hospital as well as community acquired infections.[1,2] This pathogen has a diverse mode of transmission, and hence can cause infection in different anatomical sites of the body. Some of the incriminating sources of transmission are soil, contaminated water, food, equipments, intravenous solutions, the hands of patients and healthcare personnel.[2,3] There are reports of 9.8 to 14.6 % prevalence rates of *Proteus* infections in Ghana.[4,5]
Different species of *Proteus* are encountered in human infections; however, bacteriological diagnosis up to the identification of species is rare in many laboratories in Ghana due to the cost and special skills involved. There is therefore limited documented information relating to patients' demographics and antibiotic susceptibility levels for infections caused by the various species of *Proteus*.[6,7,8] This study seeks to determine the prevalence of the various *Proteus* infections in relation to patients' demographics and the response of the different species to commonly prescribed antibiotics at the Komfo Anokye Teaching Hospital (KATH) in Ghana.

MATERIALS AND METHODS

Isolation site

Different clinical samples such as urine, purulent material from wounds or abscesses, ear swabs, sputum, blood or aspirates (of joint fluid, pleural fluid, ascitic fluid and pus) collected from 2361 patients suspected of bacterial infection at KATH were cultured to isolate the organisms. Demographic data (such as age, sex, in-patient and out-patient status) of the patients was recorded prior to sample collection.

Cultivation and Identification

The clinical samples collected were aseptically inoculated on plates of Blood agar, Cystine-Lactose-Electrolyte-Deficient (CLED) agar and MacConkey agar (Oxoid Cambridge, UK) and incubated at 37 ^{o}C for 24 h. The morphological characteristics of the colonies including size, shape, colour, pigmentation and haemolytic nature were recorded. Suspected *Proteus* colonies were isolated and identified through biochemical tests according to Barrow and Felthan:[9] based on whether they were positive for nitrate reduction; H_2S gas production; methyl-red and urease reactions; and negative for lactose fermentation. Indole production differentiated *P. vulgaris* isolates from the other species; *P. mirabilis* and *P. penneri* were identified by maltose fermentation and ornithine decarboxylase production. *P. vulgaris* (NCTC 4175) and *P. mirabilis* (NCTC 8309) were the reference strains employed.

Antimicrobial susceptibility test

Modified Kirby-Bauer disk diffusion method[10] was used to test the susceptibility of the *Proteus* isolates to different antimicrobial agents (obtained from BDH London, UK): ampicillin (10 µg), tetracycline (30 µg), chloramphenicol (30 µg), cefuroxime (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg), gentamicin (10 µg), amikacin (10 µg) and co-trimoxazole (25 µg). The inocula were prepared by growing the various *Proteus* species on separate agar plates and colonies from the plate were transferred with inoculating loop into 3 ml of normal saline in a test tube. The density of these suspensions was adjusted to 0.5 McFarland standards. The surface of Muller-Hinton agar (Oxoid Cambridge, UK) plate was evenly inoculated with the organisms using a sterile swab. The swab was dipped into the suspension and pressed against the side of the test tube to remove excess fluid. The wet swab was then used to inoculate the Muller-Hinton agar by evenly streaking across the surface. By means of Disc Dispenser (Oxoid Cambridge, UK), the antibiotic discs were applied to the surface of the inoculated agar and the plates were incubated overnight at 37 ^{o}C. The diameter of zone of growth-inhibition observed was measured and compared to the chart provided by National Committee for Clinical Laboratory Standards (NCCLS).

β-Lactamase Production Test

Proteus isolates resistant to ampicillin were tested for beta β-lactamase production. A strip of Whatman filter paper No. 1 was placed in a sterile Petri dish and drops of buffered crystalline penicillin bromocresol purple solution were added until the paper was almost saturated. About 15 colonies of the ampicillin-resistant organism were transferred with a sterile inoculating loop and spread evenly on about 5 mm length of the filter paper. The plate was covered, incubated at 37 ^{o}C for 30 minutes and examined for a colour change. β-lactamase production was indicated by colour change from purple-blue to yellow while non β-lactamase producers showed no colour change.[10]

RESULTS

Proteus species isolated

Three *Proteus* species were recovered from 200 of the 2361 clinical samples collected (Table 1) and this gave a prevalence rate of 8.4 %. Eighty-six of these samples (43 %) were taken from male patients and 114 (57 %) from females. All the age groups had at least one species present with *P. mirabilis* being the highest (Figure 1) that could be detected among all the age groups (Table 2). *P. vulgaris* accounted for 30.5 % of the *Proteus* isolates and was present in all the age groups except the 90 - 99 years age group. *P. penneri* (8.0 %) was absent in samples obtained from < 1, 50 - 59 and 70 - 79 years age groups. Wound samples contributed the highest percentage of *Proteus* (64.5 %) followed by ear swab.

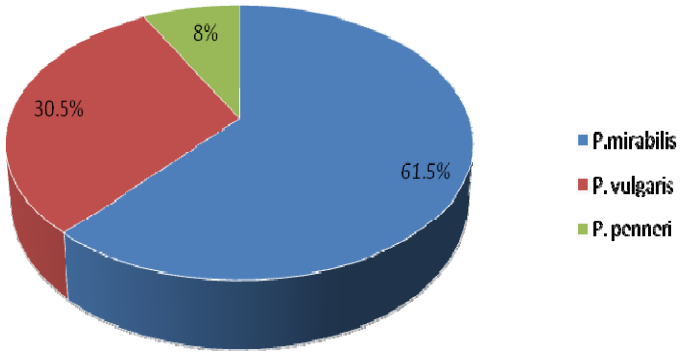

Figure 1 Distribution of the various *Proteus species* isolated

Table 1. Distribution of *Proteus species* among In- and Out-patients in relation to specimen types

Samples		In-Patients	Out-Patients	Total No. of species	Total No. of Isolates
Blood	Pm	10	2	12	
(n=853)	Pv	3	0	3	16
	Pp	1	0	1	
Wounds	Pm	18	59	77	
(n=541)	Pv	16	24	40	129
	Pp	6	6	12	
Urine	Pm	1	6	7	
(n=516)	Pv	0	0	0	7
	Pp	0	0	0	
Ear Swab	Pm	4	8	12	
(n=76)	Pv	2	11	13	27
	Pp	1	1	2	
Sputum	Pm	0	3	3	
(n=205)	Pv	0	1	1	4
	Pp	0	0	0	
Aspirates	Pm	9	3	12	
(n=170)	Pv	1	3	4	17
	Pp	1	0	1	

Key: Pm = *Proteus mirabilis*; Pv = *Proteus vulgaris*; Pp = *Proteus penneri*,
n = number of clinical specimens tested.

Table 2. Distribution of isolated *Proteus species* in relation to age

	P.mirabilis		***P. vulgaris***		***P. penneri***		**Total**	
Age	**Number**	**percentage**	**Number**	**percentage**	**Number**	**percentage**	**Number**	**Percentage**
<1	8	4	4	2	0	0	12	6
1-9	20	10	10	5	5	2.5	35	17.5
10-19	15	7.5	5	2.5	2	1	22	11
20-29	10	5	9	4.5	1	0.5	20	10
30-39	16	8	10	5	3	1.5	29	14.5
40-49	14	7	4	2	2	1	20	10
50-59	14	7	7	3.5	0	0	21	10.5
60-69	6	3	4	2	3	1.5	13	6.5
70-79	11	5.5	6	3	0	0	17	8
80-89	8	4	2	1	0	0	10	5
90-99	1	0.5	0	0	0	0	1	0.5

Antimicrobial susceptibility of the Proteus isolates

The *Proteus* isolates recovered were generally susceptible to gentamicin, amikacin, ceftriaxone, cefuroxime and cefotaxome. However, 70 – 90 % of *P. mirabilis* and *P. vulgaris* isolates exhibited resistance to ampicillin, co-trimoxazole, tetracycline and chloramphenicol while all isolates of *P. penneri* were resistant to these antibiotics (Figure 2). All the *P. penneri* isolates were resistant to at least 3 antibiotics while 93.4 % and 84.6 % of *P. vulgaris* and *P. mirablis* respectively were found to be multiple drug resistant (Table 3). Out of the 155 *Proteus species* found to be resistant to ampicillin, 113 (72.9 %) produced β-lactamase enzyme (Table 4). There was no significant difference in the β-lactamase production by the various *Proteus* species.

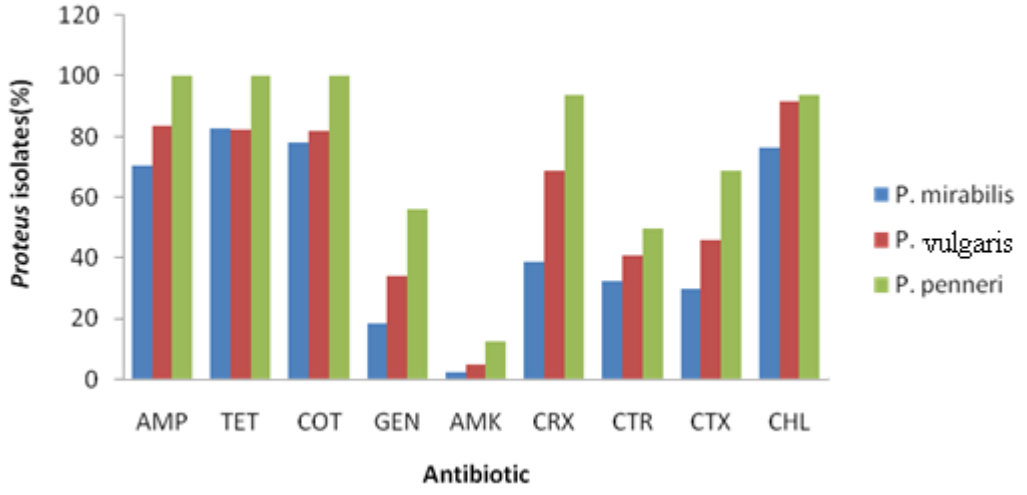

Figure 2. Antimicrobial resistant patterns of the *Proteus* isolates

Key: AMP-Ampicillin, TET-Tetracycline, COT-Cotrimixazole, GEN-Gentamicin, AMK-Amikacin CRX-Cefuroxime, CTR-Ceftriaxone, CTX- Cefotaxime, and CHL-chloramphenicol.

Table 3: Multi-Drug Resistant (MDR) *Proteus* isolates

Organisms isolated	Total isolates	No. of isolates found to be MDR	% MDR isolates
P.mirabilis	123	104	84.6
P. vulgaris	61	57	93.4
P. penneri	16	16	100
Total	**200**	**177**	**88.5**

NOTE: "Multi-drug resistance" was defined as resistance to at least 3 antibiotics

Table 4: β-lactamase production by the various *Proteus* species

Isolates	No. tested	β-lactamase positive	%	β-lactamase negative	%
P .mirabilis	87	67	77	20	23
P. vulgaris	52	34	65.4	18	34.6
P. penneri	16	12	75	4	25
Total	**155**	**113**	**72.9**	**42**	**27**

DISCUSSION

Species identification and surveillance of antimicrobial resistance is essential in management and control of infections. These practices are usually absent in most of our hospitals mainly due to the high costs involved. In this study we investigated the presence of *Proteus* species in 2361 clinical samples collected between November 2006 and July 2007 at KATH. Three *Proteus* species (*P. mirabilis*, *P. vulgaris* and *P. penneri*) were identified to be responsible for causing infections in various anatomical sites. *P. mirabilis* was the most common species isolated, accounting for 61.5 % of all the infections and hence responsible for the majority of *Proteus* infections. This result agrees with similar studies conducted in England, Wales and Northern Ireland.[11,12] Wounds recorded the highest percentage of *Proteus* isolates (64.5 %) and this confirmed the findings of Newman *et al*.[4] in Ghana and Yah *et al*.[13] in Nigeria. *Proteus* is therefore a common cause of wound infections in Ghana and other parts of West Africa. Our findings are, however, in contrast with those from Europe and Asia;[14,15] which showed *Proteus* species to be more commonly encountered in urine than in other clinical specimens.

P. mirabilis was the only *Proteus* species encountered in urine samples and this supported the finding that *P. vulgaris* and *P. penneri* infections of the urinary tract are rare [15,16,17] whereas *P. mirabilis* has a higher propensity for colonizing the urinary tract due to difference in its pathogenicity.[18] *Proteus* infections were also common among the out-patients (59 %) as compared to in-patients (41 %). This trend is similar to the finding of Chung *et al*.[15] Out of the 200 clinical specimens from which *Proteus* was recovered, 86 (43 %) were collected from males and 114 (57 %) from females. There was no significant difference ($p > 0.05$) between the males and females infected with *Proteus* in this study. The *Proteus* infections were detected in all age groups with the age group 1 – 9 years (most are below 2 years in the study, hence underdeveloped immune system) registering as the highest group infected (17.5 %).

The *Proteus species* isolated were found to have high antimicrobial resistance against tetracycline (85 %), chloramphenicol (82.5 %), co-trimoxazole (81 %) and ampicillin (77 %). Similar results were reported earlier in Ghana.[4] The high antibiotic resistance of *Proteus* may be an indication of the resistance levels among the

enterobacteriaceae and perhaps salmonellae since indiscriminate ingestion of antibiotics provides selective pressure, leading to a higher prevalence of resistant bacteria[19] which is very common in developing countries like Ghana. Not only are these species potential causes of infections but also potential reservoirs of resistance genes that could be transferred to other bacterial pathogens. The high levels of β-lactamase production and multi-drug resistance of the isolates are indications of an increase in the resistance menace reported by earlier studies[4] in Ghana.

CONCLUSION

P. mirabilis, *P. vulgaris* and *P. penneri* are the species implicated in *Proteus* infections; wounds recorded the highest incidence of *Proteus* infections at KATH. The species were susceptible to amikacin and gentamicin, and third generation cephalosporins. They were, however resistant to ampicillin, tetracycline, chloramphenicol and co-trimoxazole and hence these must not form part of the empirical antibiotics for the treatment of *Proteus* infections at KATH. β-lactamase production and multi-drug resistance have all been exhibited by the isolates. This study is therefore a step towards the generation of national data on the prevalence of antimicrobial resistant pathogens in Ghana.

Acknowledgement

We will like to acknowledge the Medical Microbiology Laboratory technicians of KATH for their assistance in this study. We also thank the Ghana government for providing funds for the study.

References

[1] Douglas G, Mandell GL, Bennett JE, Dolin R. (2000) Bennett's Principles and Practice of Infectious Diseases. 5th ed. Philadelphia, Pa: Churchill Livingstone. 9:121-126
[2] Emori GT, Gaynes RP. (1993) An overview of nosocomial infections, including the role of the microbiology laboratory, *Clin Microbiol* Rev. 6: 428.
[3] Heinzelmann M, Scott M, Lam T. (2002) Factors predisposing to bacterial invasion and infection. *Am J Surg*; 183(2): 179-90
[4] Newman MJ, Frimpong E, Asamoah-Adu A, Sampane-Donkor E. (2006) Resistance to Antimicrbial Drugs in Ghana. *The Ghanaian – Dutch collaboration for Health Research and Development*, 1-6.
[5] Ohene A. (1997) Bacterial pathogens and their antimicrobial susceptibility in Kumasi, Ghana, *East Afri. Med. J*. 74:450–455.
[6] Yao JDC, Moellering RC Jr. (1999) Antibacterial agents. Manual of clinical microbiology. 7th ed. Washington, D.C. *American Society for Microbiology*. 1474–1504.
[7] Tenssaie ZW. (2001) Multiple antimicrobial resistance in gram negative bacilli isolated from clinical specimens, Jimma Hospital, Southwest Ethiopia, *Ethiopia Med. J*. 39: 305 –312.
[8] Patterson JE, Hardin TC, Kelly C. (1999) Association of antibiotic utilization. Measurement and control of multiple drug resistance. 19(8):120-128.
[9] Barrow GI, Felthan RKA. (2003) Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd Ed. Cambridge University Press. Cambridge UK. 351-353.
[10] Cheesebrough M. (2000) District Laboratory Practice in Tropical Countries. Part 2, Cambridge University Press, UK. 132–143.
[11] Chow AW, Taylor PR, Yoshikawa TT, Guze LB. (1979) A nosocomial outbreak of infection due to multiple strains of resistant *Proteus mirabilis*: Role of intestinal colonization as a major reservoir. *J. Infect. Dis.* 139:621–627
[12] Jones R, Vincent BA, Saunders WB. (2003) Bacteraemia, England, Wales and Northern Ireland: *Commun Dis Rep CDR*. Wkly [serial online]
[13] Yah SC, Egbanfona NO, Oranusi S, Abouo AM. (2001) Widespread plasmid resistance genes among *Proteus species* in diabetic wounds of patients in Ahmadu Bello University Teaching Hospital (ABUTH) Zaria, *Afr. J. Biotechnol*. 6(15):1757-1762.
[14] Reslinski A, Gospodarek E, Mikucha A. (2005) Prevalence of multi-drug resistant *Proteus species* in clinical specimens and their susceptibility to antibiotics, *Med. Dosw. Micribial*, 57(2):175-184.
[15] Chung HI, Hsueh T, Chih T. (1999) Prevalence of *Proteus species* in urinary tract infections in a regional hospital in Trinidad. 62:438-442.
[16] Foxman B. (2000) Urinary tract infections: Incidence and risk factors. *Am. J. Pub. Health*, 80:331-333.
[17] Nawal VI, Lipsky BA, Allen AG. (1994) *In vitro* antimicrobial activities against *Proteus species* isolated from patients in a Tertiary care Hospital in Jordan: Frequency of isolation and Epidemiology. *Scientific Research and Essay*, 3(4):122-126.
[18] Mobley HLT. (1994) Virulence of *Proteus mirabilis* in urinary tract infections. Molecular pathogenesis and clinical management. ASM Press, Washington, D.C. 245–269.
[19] Levy SB. (1999) Antibiotic resistance: an ecological inbalance. *Ciba Found. Symp*. 207**:**1-14.