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EPIDEMIOLOGICAL STUDIES ON PROTEEAEE ISOLATES FROM CLINICAL SPECIMENS IN THE LAQUINTINIE HOSPITAL IN DOUALA, CAMEROON

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Abstract

The tribe Proteeae is a group of bacteria within the family *Enterobacteriaceae* and is responsible for most cases of nosocomial infections in hospital settings. The objective of this study was to determine the prevalence of members of Proteeae from clinical specimens in Laquintinie hospital in Douala. Specimens were collected from patients and screened for Proteeae using standard microbiological and biochemical methods (API 20 Enterobacteriaceae gallery). Of the 3414 clinical specimens made of 2712 urine, 264 blood, 243 CSF and 195 wounds and burns, 1136 (33.3%) yielded a positive bacterial growth, of which 230 (20.2%) were Gram positive and 906 (79.7%) were Gram negative. 164 (14.4%) isolates were identified as members of Proteeae of which 110(67.1%) were from urine, 37(22.6%) from wounds and burns, 10(6.1%) from blood and 7(4.3%) from CSF. Speciation of the Proteeae isolates showed that 111 (67.7%) were *Proteus mirabilis*, 21(12.8%) *Proteus vulgaris*, 11 (6.7%) *Providencia alcalifaciens*, 6 (3.6%) *Providencia stuartii*, 4 (2.4%) *Morganella morganii* and 5 (3.0%) *Proteus penneri* and *Providencia rettgeri*. There was a significant difference between the type of clinical specimens and the age of patients ($X^2 = 52.623$ $p < 0.05$). Most Proteeae isolates were susceptible to imipenem, ceftazidime, chloramphenicol, gentamicin, nalidixic acid, ofloxacin and amikacin. These findings have significant clinical and epidemiological implications.

Keywords: Proteeae isolates, Clinical specimens, Laquintinie Hospital, Cameroon

INTRODUCTION

The tribe Proteeae consists of a unique group of bacteria within the family *Enterobacteriaceae*. The genera include; The Enterobacteriaceae are Gram-negative, non-sporing rods measuring about $1-4 \times 0.6\mu\text{m}$. They are peritrichous and motile. Proteeae can be found in animals, soil and in the intestinal tract of humans. The Proteeae which include *Proteus*, *Providencia* and *Morganella* are unique among the *Enterobacteriaceae* in that, they have the ability to oxidatively deaminate a wide range of amino acids especially phenylalanine. *Proteus*, *Providencia* and *Morganella* are all oxidase negative and urease positive for some of their strains. They are readily isolated from pathological specimens [1]. Proteeae are of concern in that they have been involved in recent years with human infections, especially in the hospital areas. They have been readily isolated from urinary tract infections especially in patients with indwelling urinary catheters. They are thought to be responsible for almost 10% of nosocomial infections [2,3]. In addition, a number of Proteeae are often resistant to antibiotics commonly used in hospital practice and as such may facilitate their spread within this environment.

Mistry et al. [4] described high prevalence of *Proteus mirabilis*, in skin abscesses of the maxilla, which had potential implications for selection of antimicrobial therapy. A case of suppurative pericarditis caused by *Proteus mirabilis* and *Citrobacter diversus* were also identified [5] even though polymicrobial gram-negative infections in cases of pericarditis are known to be rare. *Morganella morganii* was found to be the sole potentially pathogenic bacterial species present in the faeces of some patients with diarrhea. Nosocomial infections outbreaks involving some strains of *M. morganii* are rare but are associated with serious morbidity and high mortality [1]. *M. morganii* has also been isolated from a patient with diabetic foot ulcer which resulted to a gas gangrene which was confounded with that caused by *Clostridium* [6]. *M. morganii* has also been responsible for the death of a 17 days termed neonate. Usually, it does not cause perinatal infection but will do in the immunocompromised patients and premature infants [7]. *Providencia* species occur in normal faeces and has been isolated from epidemic and sporadic cause of diarrhea in man though their importance in the causation of diarrhoeal disease

is not easy to assess. However, *Providencia rettgeri* has been associated in cases of traveller's diarrhea in Japan [8]. They also cause UIT especially *Pro. stuartii* and *Pro. rettgeri* which are often hospital acquired [9]. Additionally, *Pro. alcalifaciens* has been reported in food borne infections outbreaks [10]. Generally, *Providencia alcalifaciens* is associated with infections of the intestinal tract and *Providencia stuartii* and *Providencia rettgeri* with infections of the urinary tract although exceptions do occur [1].

Epidemiological data on Proteae isolates in clinical specimens and their antimicrobial sensitivity testing is important to help clinicians in the empirical selection of medications. In Cameroon, such data are scarce in healthcare settings due to dwindling resources and information used is usually from developed countries. The objective of this study was therefore to determine the prevalence of infections caused by organisms of the tribe *Proteae* from clinical specimens in Douala Laquintinie hospital.

MATERIALS AND METHODS

Study design and subjects

This study was conducted in the Douala Laquintinie hospital, Cameroon, a tertiary health care hospital providing a full range of surgical, medical and super speciality facilities. The study was carried out from January to June 2010. All the subjects were patients sent to the bacteriology laboratory by physicians. There was no formal definition and recruitment was at the discretion of the attending physicians. However, prior to specimen collection, patients were explained the purpose of the study and made to understand that it was not a hospital obligation to participate in the research and neither was it a pre-requisite to accessing any hospital services publicly available. Patients' consent to participate was solicited. Ethical clearance was obtained from the Regional Delegation of Public Health.

Bacteriological analysis

Smears of the specimens were made and Gram-staining was also done to have an idea of the expected bacteria be it rod or cocci. For the urine samples, wet mount was made using the Mallassez counting chamber. Five bands were counted and multiplied by two to get the number of pus cells per cubic millimeter (mm^3). Specimens that had less than 5 pus cells/ mm^3 cells were not cultured and those above were cultured. Specimens were plated on cysteine-lactose-electrolyte-deficient (CLED) agar, Eosin-methylene blue (EMB) and blood agar. After overnight incubation at 37°C, a representative of each type of oxidase Negative Non-lactose

fermenting colony from the EMB and CLED agar were subcultured into peptone water and incubated at 37°C for a few hours. These were further inoculated on phenylalanine and urease agar slants and incubated overnight at 37°C. Proteae isolates changed the phenylalanine agar to green. Further test were carried out for full identification. For blood Culture, about 10ml of patient's blood was added to Brain Heart Infusion Broth (BHI). The broth was incubated at 37°C and observed for growth on the 1st, 2nd, 3rd, 5th, 8th, 16th and 21st day. When positive growth was observed the broth was subcultured into blood and EMB agar.

All cultured plates were observed for colonial morphology of the bacteria such as size on agar, blood agar plates were used to detect swarming and hemolysis. The characteristic smell of Proteae isolates was also noted. Oxidase test were performed on the non-lactose fermenting colonies using filter paper impregnated with a freshly prepare 10% solution of tetramethyl-p-phenylene-diamine dihydrochloride. Proteae isolates were purified alternatively on EMB agar before preservation. Purified colonies were maintained on nutrient agar slants at 4°C until used. Isolates were identified by their biochemical characteristics using the API 20 Enterobacteriaceae gallery (Biomérieux, France) as described by Cheesbrough[11].

The sensitivity of each identification test medium was confirmed by inoculating one or two uninoculated tubes of the batch alloy with the test medium inoculated with positive and negative control organisms. For the positive control, one of the test medium was inoculated with a stock culture of a bacterium (e.g a phenylalanine deaminase positive *Proteus* species) to give a positive reaction and another tube with stock culture known to give a negative reaction (e.g *Escherichia coli*). These positive and negative controls were incubated at 37°C and examined along site the alloy.

Antibiotic susceptibility testing

Single antibiotic impregnated disk were used with the following concentrations; amikacin (10µg), imipenem (10µg), ciprofloxacin (5µg), clotrimoxazole(30µg), tobramycin(30µg), chloramphenicol (30µg), ceftazidime (10µg), gentamicin (10µg), amoxycillin(30µg), ofloxacin (10µg), and nalidixic acid (30µg). Four to five colonies of identical morphology were emulsified into 3-4ml of sterile distilled water. The inoculum was prepared to meet the 0.5 McFarland standard (approximately 10^8 microorganism per ml), (PML Microbiologicals, Inc, 2001). The prepared Mueller-Hinton agar plates were inoculated by streaking method using a swab of

the inoculums. The plates were allowed to dry for 5 minutes to remove excess moisture. The antibiotic discs were then firmly placed on the inoculated plates using a flame sterile forceps. The discs were evenly distributed to allow an edge not less than 15 mm from the walls of the petridish. The inoculated plates were inverted and incubated aerobically at 37°C for 24hours. After overnight incubation, each plate was examined and the diameter of the complete inhibition zones were noted and measured with a caliper in millimeter. The inhibition zones were interpreted using the manufacturers standard table as being; susceptible (sensitive), intermediate or resistant.

A control test was done for the cultures since disc diffusion test vary with a number of experimental conditions and the organism in question. The control culture (*E. coli*) was plated simultaneously with the test culture and on separate plates. The zones of inhibition produced in the cultures by the same discs were compared.

Statistical analysis

Chi-square and t-test were used to test for the significance of the results obtained. $p < 0.05$ was considered statistically significant.

RESULTS

All Proteeae isolates were phenylalanine positive. Only the *Proteus* isolates swarmed on blood and EMB agar at 37°C though a few strains did not. Of the urease positive isolates, the test results could be easily observed within 6 hours. Only *P. mirabilis* were indole negative. Some *P. vulgaris* strains were salicin and esculin positive. All isolates were oxidase negative, non-lactose fermenting colonies and gram negative rods.

A total of 3414 specimens were received from the hospital service areas of; Medical, Surgery, Pediatrics, Out-patients, Obstetrics and Gynaecology. Distribution of the clinical specimen by service area is shown in Table1. The largest number of wounds and burns were from the surgery wards, while most urine samples were from the out-patients and pediatrics services. There was no blood and cerebrospinal fluid (CSF) samples from obstetrics and gynaecology services.

TABLE 1: DISTRIBUTION OF CLINICAL SPECIMENS BY AREA OF MEDICAL SERVICE

Service	Laboratory findings	Urine	Blood	Wounds and burns	CSF	Total
Surgery	Samples	57	12	96	9	174
	Bacterial isolates	39	3	72	6	120
	Proteeae	20	1	17	3	41
Medicine	Samples	360	102	36	75	573
	Bacterial isolates	177	36	12	18	243
	Proteeae	36	0	9	0	45
Pediatrics	Samples	726	126	6	108	966
	Bacterial isolates	324	12	5	20	361
	Proteeae	36	9	2	3	50
Obstetrics and Gynaecology	Samples	150	0	12	0	162
	Bacterial isolates	27	0	10	0	37
	Proteeae	6	0	3	0	9
Out -patients	Samples	1419	24	45	51	1539
	Bacterial isolates	339	3	30	3	375
	Proteeae	12	0	6	1	19
Total		2712	264	195	243	3414

Table 2 shows the distribution of bacterial isolates by clinical source. There were 1136 bacterial isolates of which 230 (20.2%) were Gram positive and 906 (79.7%) were Gram negative. Of the Gram negative bacteria, 164 (18.1%) were Proteeae isolates. The greatest number of bacterial isolates was from urine followed by

wounds and burns. *Staphylococcus aureus* accounted for 114 (49.6%) of the gram positive isolates. Most of the 18.1% Proteeae were from urine followed by wounds and burns then blood specimens. There was a significant difference between the bacterial species and the site of recovery, ($X^2 = 396.79$ $p < 0.005$).

TABLE 2: CLINICAL SPECIMENS' SOURCE AND DISTRIBUTION OF BACTERIAL ISOLATES

Bacterial isolates	No. of isolates	No. of bacteria isolates from			
		Wounds and burns	Urine	CSF	Blood
<i>Staphylococcus aureus</i>	114	11	84	8	11
<i>Staphylococcus spp</i>	65	0	65	0	0
<i>Streptococcus spp</i>	51	6	27	15	3
<i>Klebsiella spp</i>	156	12	134	7	3
<i>Escherichia coli</i>	453	18	427	0	8
<i>Salmonella spp</i>	23	9	5	0	9
<i>Enterobacter spp</i>	39	6	33	0	0
Proteaeae	164	37	110	7	10
Uncharacterized species	71	30	21	10	10
Total	1136	129	906	47	54

$X^2 = 396.79$ $p < 0.005$

Table 3 shows the frequency of isolation of Proteeae species from clinical specimens. Of the 164 Proteeae isolates, 111 (67.7%) were made of *P. mirabilis* 21(12.8%) of *P. vulgaris*, 11 (6.7%) of *Providencia. alcalifaciens*, 6 (3.6%) of *Pro. stuartii*, 4 (2.4%) of *Morganella morganii* and 5 (3.0%) of *P. penneri* and *Pro. rettgeri* respectively. There was no significant difference between the species of

Proteeae isolated and the clinical specimens ($X^2 = 17.63$, $p > 0.05$). The Distribution of Proteeae isolates by age in relation to clinical source is shown in Table 4. A greater number of Proteeae isolates were from the elderly aged 50 and above followed by the younger aged 0 - 10 years, ($X^2 = 52.623$ $p < 0.05$).

TABLE 3: FREQUENCY OF ISOLATION OF PROTEEAE SPECIES FROM CLINICAL SPECIMENS

Source	No of positive bacteria isolates	No (%) of Proteeae isolates	Proteeae isolates identified						
			<i>P. mirabilis</i>	<i>P. vulgaris</i>	<i>P. penneri</i>	<i>Pro. Stuartii</i>	<i>Pro. alcalifaciens</i>	<i>Pro. rettgeri</i>	<i>M. morganii</i>
Wounds and burns	129	37 (28.7)	22	7	0	2	3	1	2
Urine	906	110 (12.1)	78	10	5	4	7	4	1
Blood	54	10 (18.5)	7	1	0	0	1	0	1
CSF	47	7 (14.9)	4	3	0	0	0	0	0
Total	1136	164 (14.4)	111	21	5	6	11	5	4

$X^2 = 17.63$ $p > 0.05$

TABLE 4: DISTRIBUTION OF PROTEEAE ISOLATES BY AGE AND CLINICAL SPECIMENS

Age (years)	Wounds and burns	Blood	Urine	CSF	Total
0- 10	4	9	55	6	74
11 - 20	6	0	3	0	9
21 - 30	6	1	2	0	9
31 - 40	3	0	1	0	4
41 - 50	8	0	15	0	23
> 50	10	0	34	1	45
Total	37	10	110	7	164

$X^2 = 52.623$ $p < 0.05$

Table 5 shows the distribution of Proteeae species isolates by age of infected patients. In all, *P. mirabilis* accounted for 111 (67.7%) of Proteeae species isolated. There was a significant level of association between age of patients and species distribution ($X^2 = 47.13$ $p < 0.05$).

P. penneri isolates were recovered mostly from the patients aged 0 - 10 years. A greater number of *Pro. rettgeri* isolates were in the infected patients of age greater than 50.

TABLE 5: DISTRIBUTION OF PROTEEEAE SPECIES ISOLATES BY AGE OF PATIENTS

Age (years)	No of isolates	<i>P. mirabilis</i> (%)	<i>P. vulgaris</i>	<i>P. penneri</i>	<i>Prv. stuartii</i>	<i>Prv. alcalifaciens</i>	<i>Prv. rettgeri</i>	<i>M. morganii</i>
0-10	74	48 (64.9)	7	5	3	8	1	2
11 -20	9	4 (44.4)	3	0	1	1	0	0
21 -30	9	3 (33.3)	4	0	1	0	1	0
31 -40	4	2 (50)	2	0	0	0	0	0
41 -50	23	15 (65.2)	5	0	0	2	0	1
> 50	45	39 (86.7)	1	0	1	0	3	1
Total	164	111	22	5	6	11	5	4

$X^2= 47.13$ $p<0.05$

Table 6 shows the frequency of Proteeae isolation from UTI's by service areas. From the surgery service, 20 (51.3%) of Proteeae isolates caused urinary tract infections. Less Proteeae UTI's were noted in out-patients 12 (3.5%) there were no marked significance of UTI's caused by Proteeae with respect to those caused by other bacteria in relation to various service areas ($t= 1.43$ $p>0.05$).

Table 7 shows the pattern of antimicrobial resistance among Proteeae isolates. Resistance to Tobramycin (TOB), Chloramphenicol (CMP), Gentamicin (GEN), and Nalidixic acid (NAL) were almost uniform. *Prv. alcalifaciens*, *P. mirabilis* and *P. vulgaris* presented some resistance to all the antimicrobial agents. There was marked resistance in *Prv. rettgeri*, *M. morganii* and *Prv. stuartii*, ($X^2= 117.12$ $p<0.005$).

TABLE 6: FREQUENCY OF PROTEEEAE ISOLATION FROM URINARY TRACT INFECTIONS BY MEDICAL SERVICE AREA

Service	No of bacterial UTI cases	No (%) of Proteeae
Surgery	39	20 (51.3)
Medicine	177	36 (20.3)
Paediatrics	324	36 (11.1)
Obstetrics and Gynaecology	27	6 (22.2)
Out - patients	339	12 (3.5)
Total	906	110 (12.1)

DISCUSSION

In the listing of gram-negative bacteria responsible for nosocomial infections, members of the tribe *Proteeae* are inclusive. The presence of *Proteeae* in clinical specimen is of great interest since like other Enterobacteriaceae they are opportunistic and may cause morbidity and motility [1].

In this study, gram-negative bacteria accounted for 79.7% of all isolates from clinical specimens examined and cultures in the medical microbiology laboratory. Gaynes and Edwards [12] in their surveillance study on nosocomial infections caused by gram-negative bacteria in intensive care units from a study period of 1986 to 2003, found that gram negative bacteria caused 23.8% of blood stream infections, 33.8% of surgical site infections and 71.1% of urinary tract infections.

Clinical findings from this research also showed that after *E. coli*, *Proteeae* were the second group of bacteria recovered in this study. *E. coli* being the major bacteria species isolated is probably due to the existence of *E. coli* as a normal intestinal flora making its colonization of other parts of the body easier especially in cases of immune suppression. This is in agreement with previous findings [13] that *E. coli* accounts for the largest cause of cystitis, prostates and pyelonephritis in hospitals acquired infections. In another study [1], *Proteus* ranked third as the case of nosocomial infections in hospitals. From another study by De Champ et al.[14] *Proteus* ranked second after *E. coli* among enterobacteria responsible for nosocomial infections. In the present study, members of the tribe *Proteeae* were mostly recovered from urine samples. They have always been recognized as important cause of nosocomial urinary tract infections associated with long-term catheterization [13].

TABLE 7: PATTERN OF ANTIMICROBIAL RESISTANCE AMONG *PROTEEA*E ISOLATES

Proteae species	TSU	GEN	AMK	TOB	NAL	OFX	CIP	AMO	IMI	CAZ	CMP
<i>P. mirabilis</i> (111)	72 (65)	33 (30)	15 (13)	80 (72)	27 (24)	30 (27)	47 (42)	43 (39)	13 (12)	63 (57)	68 (61)
<i>P. vulgaris</i> (21)	12 (57)	12 (57)	8 (26)	9 (42)	15 (71)	16 (76)	15 (71)	21 (100)	3 (14)	4 (19)	19 (90)
<i>P. penneri</i> (5)	1 (20)	1 (20)	5 (100)	5 (100)	4 (80)	0	0	5 (100)	3 (60)	1 (20)	5 (100)
<i>Pro. stuartii</i> (6)	1 (17)	6 (100)	1 (17)	6 (100)	4 (67)	3 (50)	2 (33)	0	3 (50)	2 (33)	6 (100)
<i>Pro. alcalifaciens</i> (11)	9 (82)	6 (55)	10 (91)	8 (73)	7 (64)	7 (64)	9 (82)	11 (100)	3 (27)	3 (27)	10 (91)
<i>Pro. rettgeri</i> (5)	1 (20)	4 (80)	4 (80)	5 (100)	5 (100)	4(80)	5 (100)	5 (100)	2 (40)	0	5 (100)
<i>M. morgani</i> (4)	4 (100)	2 (50)	2 (50)	2 (50)	4 (100)	4 (100)	2 (50)	4 (100)	0	0	4 (100)

$\chi^2=117.12$ $p<0.005$ AMK, Amikacin; IMI, Imipenem; CIP, Ciprofloxacin; TSU, Clotrimoxazole; TOB, Tobramycin; CMP, Chloramphenicol; CAZ, Ceftazidime; GEN, Gentamicin; AMO, Amoxycillin; OFX, Ofloxacin; NAL, Nalidixic acid.

The predominance of *Proteus* among all the Proteae is consistent with previous reports [1, 14]. Apart from urine specimens being the source of most clinical isolates of Proteae, others were recovered from wounds and burns, followed by blood and the least in CSF. *P. mirabilis* was recovered more often than *P. vulgaris* especially in UTIs. The predominance of *P. mirabilis* in UTIs is related to its specific pathogenic ability. It has the potentials to colonize the urinary tract by tolerating the local pH and swarm by the aid of fimbriae. *P. mirabilis* also has the ability to evade host defenses by producing IgA which degrade protease and finally damaging the host tissue with urease and hemolysins [15,16,17]. According to the Center for Disease Control [18], *Proteus* is responsible for 3% of nosocomial infections in American hospitals.

From this study the significance on the distribution of Proteae isolates related to age was explicit. Recovery of Proteae isolates was mostly from urine of patients aged 0- 10 and > 50 years. The young have a less developed immune system and for the elderly a system that wears out with time. Age specific infections rate could also be due to the fact that *P. mirabilis* mostly infects host compromised by underlying diseases or abnormalities. The elderly patients have increased risk factors predisposing them to infections by Proteae. Risk factors like impaired

host defense which occurs in cases of underlying diseases. Though macrophages and polymorphonuclear leukocytes functions are not affected by age, the quality and quantity of antibodies produced by B-cells are jeopardized thus increasing susceptibility to bacterial infections.

CSF and blood are generally sterile in nature, but the presence of any microbe is indicative of infection and polymicrobes implies contamination during laboratory culture procedures. Isolation frequency of Proteae isolates from blood samples of patients according to age revealed that patients aged 0-10 years had the highest rate of infection. Neonates are generally easily infected by various bacteria at birth. This is due to the nature of the vagina at pregnancy which favors replication of bacteria. As such during birth the neonate becomes infected at the level of the birth canal. Ovalle et al. [7] reported *M. morgani* to be the cause of sepsis in premature infant and a cefotaxime resistant sepsis in a 17 days neonate infected at birth. Other factors could have well been responsible for sepsis in patients age 0-10 years caused by Proteae.

Proteae bacteremia in the elderly (>50) may be associated to operative procedures on the lower urinary tract which requires the use of urinary catheters. Bacteremia may be provoked

immediately after surgery or by delayed removal or change of the catheter. Patients with long-term indwelling urinary catheters usually have bacteriuria and sometimes bacteremia[13]. Underlying urethral abnormalities, type of catheter and bacteriocins in urine may have an influence on bacteremia [19].

From this study, *Providencia* species were responsible for 13, 6% of urinary tract infections which is comparatively less than that proposed (18%) in a Canadian study [9]. *Providencia* bacteriuria in the hospital settings of the united states are rare that is; 0.3- 1% which denotes a good reduction in their cause of nosocomial infections [20]. Also Koreishi et al. [21] have shown that *Providencia* spp could be responsible for 6.33% of blood stream infections. But from this study 10% *Providencia* were recovered from patients with blood sepsis, which however was not significant.

In this study, from the four *M. morgani* isolates recovered two were from wounds and burns and the others from urine and blood. Recovery of *M. morgani* in wounds and burns is consistent with previous findings [6] in which this organism was isolated from a diabetic foot ulcer. *M. morgani* infections are uncommon but easily observed in HIV/AIDS patients, neonates and the elderly. The significant relationship between Proteae species and the age range group in this study shows that there was a high percentage of *P. mirabilis* from patients age 0-10 years followed by those >50 years. This is in accord with another report [1]. *P. penneri* was obtained in urine of patients aged 0-10 years; and it was probably due to contamination of the urinary tract by fecal residues, since *P. penneri* had always, in the past, been associated with outbreaks of diarrheal infections and kidney stones formation. The high rate of recovery of Proteae from patients in the surgery service and urinary tract infections explains the fact that most patients may have just been cross contaminated. Infection may have resulted from improperly sterilized equipments, contaminated sterile surgery material, infected personnel and environmental factors such as bedding and post- surgery catheters.

In this study, of a total of 1539 samples obtained from out-patients, only 19 (0.01%) Proteae isolates were recovered. This indicates that Proteae species in out-patients may have also resulted from specimen contamination or patients' contamination on their passage in the consultation room. Such assumption suggests the possibility of cross-infection presumably from contaminated or unsterilized equipments. Most Proteae isolates obtained in this study where susceptible to imipenem, ceftazidime,

chloramphenicol, gentamicin, nalidixic acid, ofloxacin and amikacin. *P. mirabilis* and *Pro. alcalifaciens* were readily distinguished by their marked susceptibility to the eleven antimicrobials. *Pro stuartii* was found to be 100% resistant to gentamicin, tobramycin and chloramphenicol but had marked susceptibility to ciprofloxacin and ceftazidime. Spach et al.[22] explained that there have been decrease in the susceptibility of ciprofloxacin due to its routine usage. Prominent resistance was also observed in *Pro. rettgeri* to tobramycin, nalidixic acid, ciprofloxacin, amoxicillin and chloramphenicol. This observation is in conformity to findings from O'Hara et al. [1]. According to this author, *Pro. rettgeri* resistance to tobramycin and gentamicin could be attributed to excessive usage of these drugs. Biedenbach et al. [23] also studied the susceptibility of Proteae organisms to gentamicin, imipenem, ciprofloxacin, tobramycin and found similar the results with those of this study.

To determine the epidemiological patterns of Proteae infections in the different service areas, Dienes test for colony compatibility was done. Strains involved in cross-infection in the surgery, medicine and out-patients department were detected. Results suggested that Dienes reactions could be due to bacteriocin- like substances. Dienes phenomenon can easily be observed when clinical isolates are labeled with fluorescent proteins, but however can be mediated by UV light or bacteriocins [24]. Dienes compatibility strains are to some extent related. Colony compatible strains observed in this study could have been due to case to case transmission as a result of inadequate nursing techniques in these service areas. Dienes positive reactions could be taken as indication that the strains were different while Dienes negative reactions should not be taken as a reliable test of identification. However Dienes reaction was the criterion used to determine strains involved in nosocomial cross infection in this research.

Lack of full information on patient's history (sex, duration of hospitalization, medical prescription and clinical syndrome) and poor specification on urine sample collection are some of the limitations of this study. Knowledge on catheterized and non-catheterized urine would have been an essential parameter for prevalence studies.

Conclusion

There was variability in the frequency of isolation of Proteae from clinical specimens. In this study, the Proteae isolates were obtained from urine, wounds and burns, blood and CSF. Of the bacterial isolates obtained from specimens in this

research, 18.1% were caused by Proteae species of which *P. mirabilis* was predominant, followed by *P. vulgaris* and *Pro. alcalifaciens*. *P. penneri* are rarely isolated from clinical specimens but in this research five were isolated. Most Proteae isolates were obtained from urine clinical samples. Epidemiological patterns of Proteae infections in the service areas showed that most isolates were from in-patients with the least from out-patients. High prevalence of Proteae infections was probably due to the extended time of hospitalization and the overcrowding nature of the wards. All Proteae isolates from this study exhibited resistance to antibiotics commonly used in hospitals. However, they were susceptible to ceftazidime, ofloxacin, nalidixic acid, ciprofloxacin and gentamicin. *Pro. stuartii*, *Pro. rettgeri*, *P. vulgaris* were readily resistant to

antibiotics comparatively to *P. mirabilis* and *Pro. alcalifaciens* which were easily susceptible to antimicrobials. *M. morgani*, *Pro. stuartii* and *P. penneri* were the Proteae most resistant to antimicrobials.

There was evidence of nosocomial cross-infection especially in the medical and surgical services of the hospital. The control of nosocomial infections caused by Proteae requires constant surveillance on their epidemiological profile and the routine usage of antimicrobials in hospitals.

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