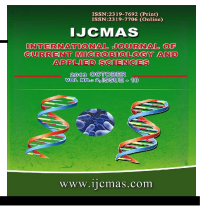
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 10 (2013) pp. 253-261**
http://www.ijcmas.com



Original Research Article

Prevalence of *Proteus* species in clinical samples, antibiotic sensitivity pattern and ESBL production

Jitendra Kumar Pandey[1]*, Akanksha Narayan[2] and Shikhar Tyagi[3]

Department of Microbiology, MGM Medical College and Hospital, Sector-18, Kamothe, Navi Mumbai- 410209, Maharashtra, India
[2]Department of Microbiology, Doon (PG) Paramedical College & Hospital, Chakrata Road, Dehradun, Uttarakhand, India
[3]Department of clinical Research, Jamia Hamdard University, New Delhi, India
Corresponding author

Keywords

Antimicrobial susceptibility; antibiotic resistance; β-lactamase.

A B S T R A C T

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 MGM Hospital, Navi Mumbai , their antibiotic resistance pattern and how they relate to patients' demographic data.

Introduction

Proteus species are among the commonly implicated pathogens in hospital as well as community acquired infections (Douglas, 2000; Emori, 1993). 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 (Emori, 1993; Heinzelmann, 2002). There are reports of 9.8 to 14.6% prevalence rates of *Proteus* infections in MGM (Newman, 2006; Ohene, 1997).

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* (Yao, 1999; Tenssaie, 2001; Patterson, 1999). 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 MGM Medical College, Navi Mumbai

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 4995 patients suspected of bacterial infection at MGM 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: [Barrow, 2003] 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 (Cheesebrough, 2000) 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

Extended spectrum Beta lactamases (ESBLs) are plasmid mediated beta lactamases capable of hydrolyzing cephalosporins and monobactams (aztreonam) but are inhibited by beta lactamase inhibitors.

Screening Tests

The isolate is a potential ESBL producer if the Zone of inhibition around the

following is :

Ceftazidime (30µg) ≤ 22mm
Ceftriaxone (30µg)≤ 25 mm
Cefotaxime (30µg) ≤ 27mm
Cefpodoxime (10µg) ≤ 17mm

Disc Approximation Test

Using Amoxyclav (Ac), Ceftriaxone and Ceftazidime

Procedure

Muller Hinton Agar was prepared and Lawn culture of test organism was prepared and inoculated antibiotic disc were placed in the centre and both the cephalosporin discs at a distance of 25mm on either side of Antibiotic.

Interpretation

Extension of edge of inhibition zone of cephalosporin towards Ac indicates potential ESBL Producer

Confirmatory Tests

ESBL double disc synergy test.

Procedure

Muller Hinton Agar was prepared and Lawn culture of test organism was prepared and inoculated antibiotic disc were placed in the centre (Caz disk and Caz + Cac disc) and incubated overnight at 37^{o} C.

Interpretation

The test organism is considered an ESBL producer if the zone size around the ceftazidime plus clavulanic acid disk is increased >5 mm vs the third generation cephalosporin (Ca) disk alone.

Result and Discussion

***Proteus* species isolated**

Three *Proteus* species were recovered from 56 of the 4995 clinical samples collected (Table 1) and this gave a prevalence rate of 1.12%. 38 of these samples (67.85 %) were taken from male patients and 18 (32.14 %) from females. All the age groups except 90-99 years age group had at least one species present. P. mirabilis being the highest with 57.14% (Figure 1) that could be detected among all the age groups (Table 2) except <1 years old and 90-99years old age groups. P. vulgaris accounted for 33.92 % of the *Proteus* isolates and was present in all the age groups except 1-9 years, 80-89 years and 90 - 99 years age group. P. penneri (8.92 %) was absent in samples obtained from < 1years, 1-9 years, 10-19 years, 30-39 years, 50 - 59 and 90 - 99 years age groups. Wound samples contributed the highest percentage of *Proteus* (67.85%) followed by urine.

Antimicrobial susceptibility of the *Proteus* isolates

The *Proteus* isolates recovered were highly susceptible to Cefotaxime, Ofloxacin, Gentamycin, Amikacin, Lomefloxacin, Ciprofloxacin and Cefaperazone . However, 44.64% of *Proteus* isolates exhibited resistance to ampicillin, 25% to Netilline and 21.42% each Cefuroxime and Pefloxacin(Figure 2). 4 out of 5 P. penneri isolates were resistant to at least 3 antibiotics while 28.12 % of P. mirablis and 36.84 % of P. vulgaris were found to be multiple drug resistant.

"Multi-drug resistance" was defined as resistance to at least 3 antibiotics. All the

Table.1 Prevalence of *Proteus* in clinical samples.

Types of Specimens	No. of Samples	No. of Proteus isolated	%
URINE	1698	11	0.647
PUS	899	38	4.22
BLOOD	1423	2	0.14
ET	180	1	0.55
SPUTUM	772	1	0.12
EAR SWAB	23	3	13.04
BODY FLUIDS	385	0	0
TOTAL	5380	56	1.05

Table.2 Age wise distribution of isolated *Proteus* species out of 56

Age Groups	*Proteus mirabilis*	*Proteus vulgaris*	*Proteus penneri*	Total out of 56	%
<1 Year	0	1	0	1	1.78
1-9 Year	1	0	0	1	1.78
10-19 Year	3	2	0	5	8.92
20-29 Year	2	2	1	5	8.92
30-39 Year	5	2	0	7	12.5
40-49 Year	6	2	1	9	16.07
50-59 Year	4	4	0	8	14.28
60-69 Year	7	5	1	13	23.21
70-79 Year	3	2	1	5	8.92
80-89 Year	1	0	1	2	3.57
90-99 Year	0	0	0	0	0
TOTAL %	32/56(57.14%)	20/50(33.92%)	5/56(8.92%)		

Figure.1 Species distribution

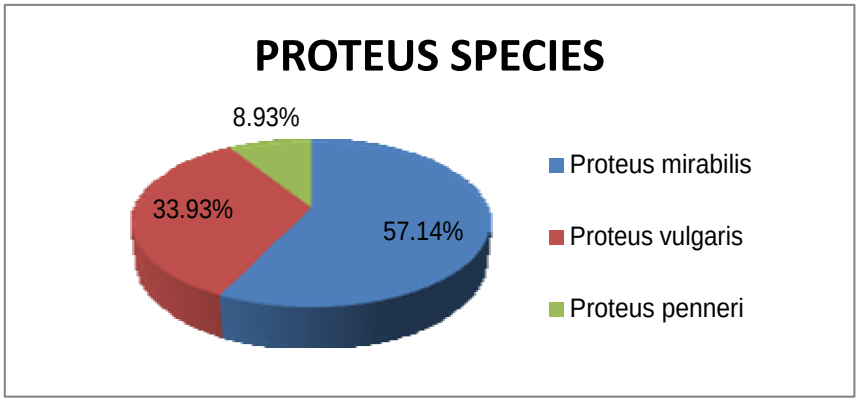


Table.3 Distribution of *Proteus* species among In-patient and Out-patient in relation to specimen type

Samples&No Studied	*Proteus* Species	In- Patients	Out-Patients	Total No. of Species	Total No. of Isolates
Pus (n=899)	Pm	19	5	24	38
	Pv	8	3	11	
	Pp	2	1	3	
Urine (n=1698)	Pm	3	1	4	11
	Pv	4	2	6	
	Pp	0	1	1	
Sputum (n=772)	Pm	0	1	1	1
	Pv	0	0	0	
	Pp	0	0	0	
ET (n=180)	Pm	0	0	0	1
	Pv	0	0	0	
	Pp	1	0	1	
Ear Swab (n=23)	Pm	1	1	2	3
	Pv	1	0	1	
	Pp	0	0	0	
Blood (n=1423)	Pm	0	1	1	2
	Pv	1	0	1	
	Pp	0	0	0	
		40/56 (71.42%)	16/56 (28.57%)	56	56

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

Figure.2 Showing antibiotic sensitivity pattern of *Proteus vulgaris*.

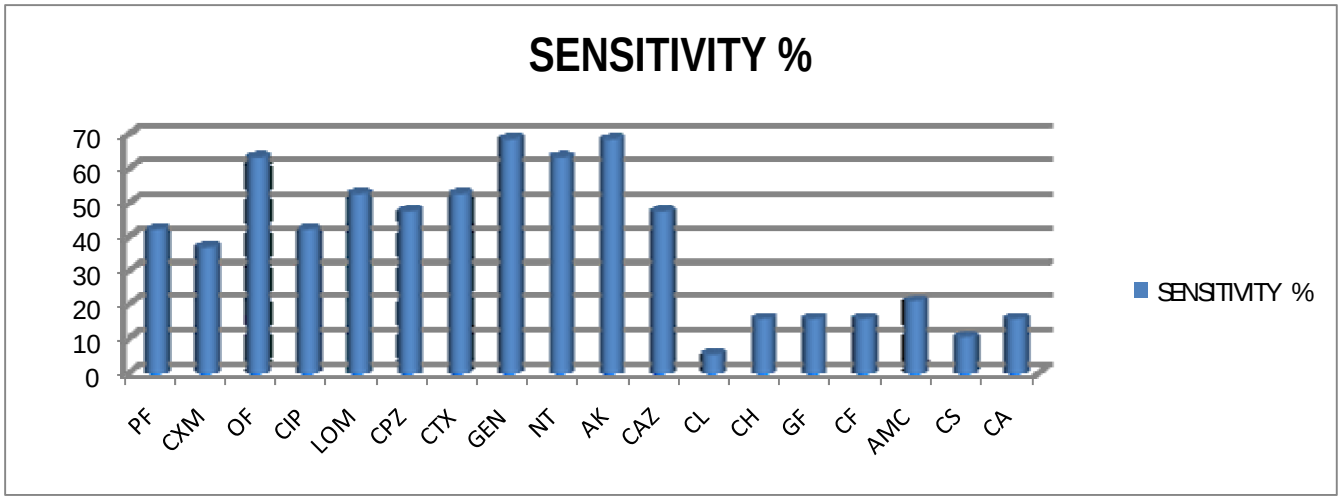


Figure.3 Shows antibiotic sensitivity pattern wise distribution of *Proteus penneri* in clinical samples

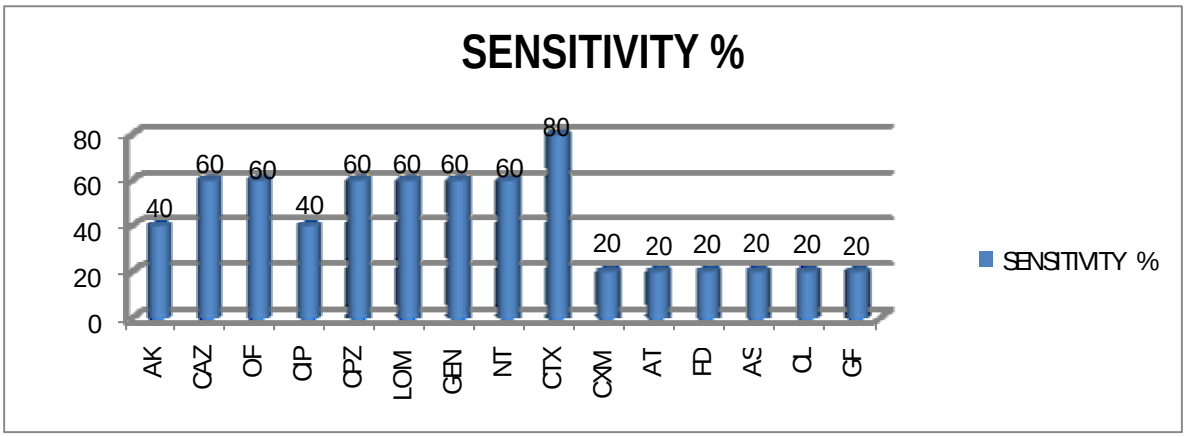


Figure.4 Showing Antibiotic sensitivity pattern of *Proteus mirabilis*

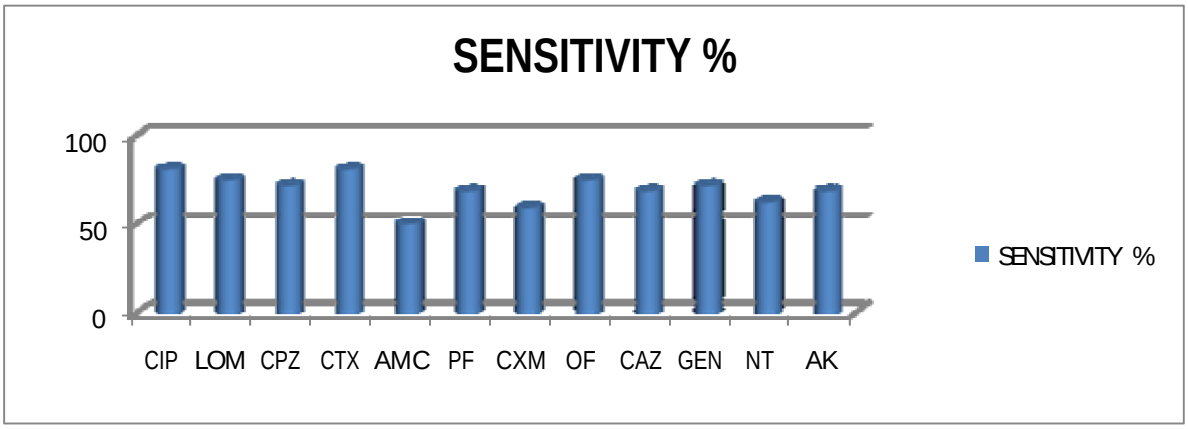


Figure.5 Multi-Drug Resistant (MDR) of *Proteus* isolates

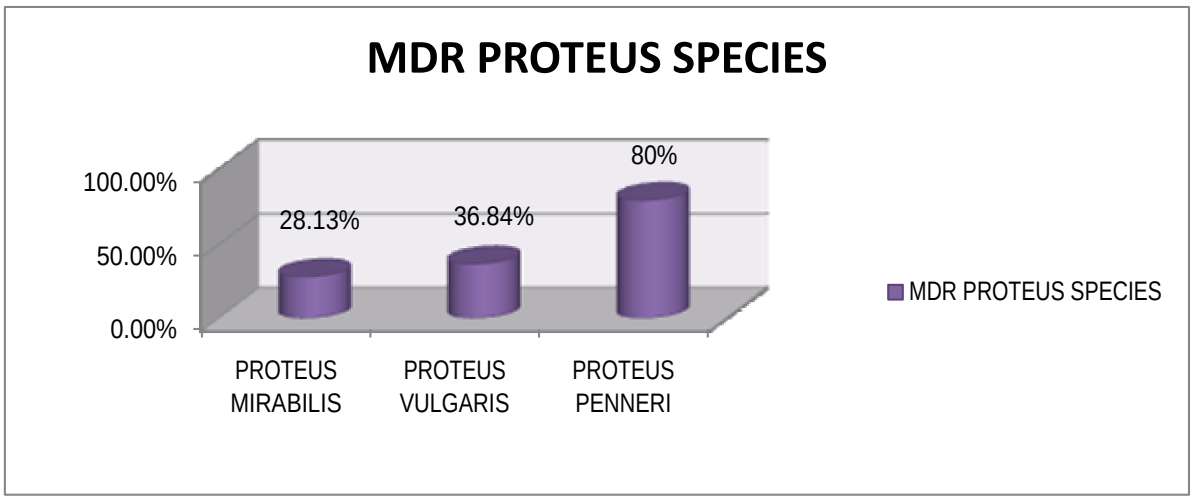
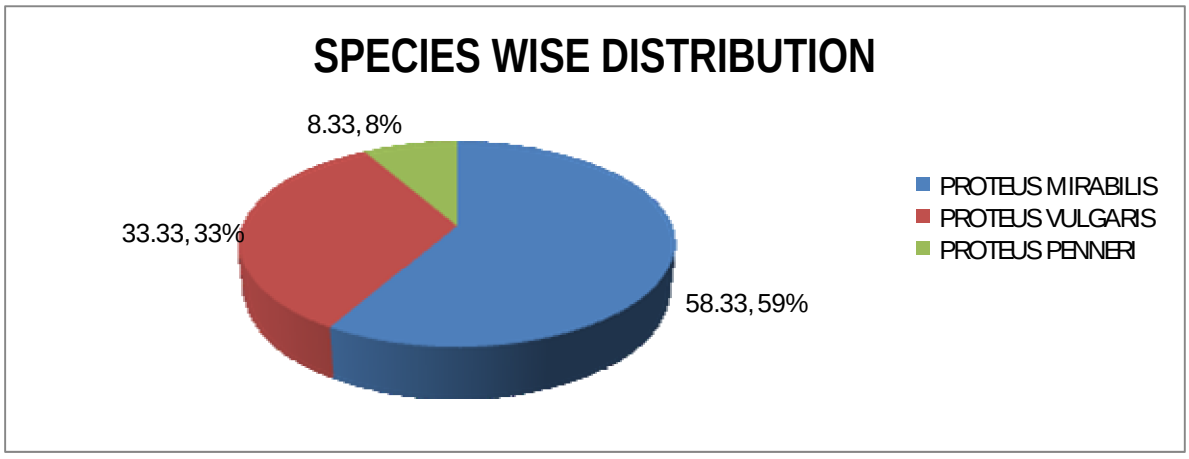


Figure.6 Species wise distribution of ESBL producing *Proteus* strain



three *Proteus* species were found to be multi-drug resistant. *Proteus penneri* showed the maximum MDR with 80% followed by *Proteus vulgaris* with 36.84% and then *Proteus mirabilis* with 28.13%.

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 4995 clinical samples collected between January 2012 and December 2012 at MGM Hospital. 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 57.14 % 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 [Chow, 1979; Jones, 2003]. Wounds recorded the highest percentage of *Proteus* isolates (67.85 %) followed by urine (19.64%). *Proteus* is therefore a common cause of wound infections in Navi Mumbai and other parts of Maharashtra, India. Our findings thus partially supports the findings of those from Europe and Asia; [Reslinski, 2005; Chung, 1999] which showed *Proteus* species to be more commonly encountered in urine than in other clinical specimens.

According to our study maximum infection in urine sample was of *P.vulgaris* which encountered 54.54% which is in contrast with the finding which supports that *P. vulgaris* and *P. penneri* infections of the urinary tract are rare (Chung, 1999; Foxman, 2000; Nawal, 1994) whereas *P. mirabilis* has a higher propensity for colonizing the urinary tract due to difference in its pathogenicity (Mobley, 1994). *Proteus* infections were also common among the in-patients (71.42 %) as compared to out-patients (28.57 %). Out of the 56 clinical specimens from which *Proteus* was recovered, 38 (67.85 %) were collected from males and 18 (32.14 %) from females. The study showed a significant difference between the males and females infected with *Proteus*. The *Proteus* infections were detected in all age groups from <1 to 99 years where

60-69 years age group registering as the highest group infected (23.21 %).

The *Proteus* species isolated were found to have high antimicrobial resistance against third generation of Cephalosporin antibiotics. All the *Proteus* species showed sensitivity to Cefotaxime, Ciprofloxacin, Lomefloxacin, Cefoperazone, Cefuroxime, Ofloxacin, Ceftazidime, Gentamycin, Netilline and Amikacin.. All the three *Proteus* species were found to be multi drug resistant. 48.86% of the total clinical samples were ESBL producers. Highest being the *Proteus mirabilis* with 58.33% followed by *Proteus vulgaris* with 33.33% and *Proteus mirabilis* with 8.33%. 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 (Levy, 1999) which is very common in developing countries like India. 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 (Newman, 2006)

P. mirabilis, P. vulgaris and *P. penneri* are the species implicated in *Proteus* infections; wounds recorded the highest incidence of *Proteus* infections at MGM Hospital, Navi Mumbai. The species were susceptible to Cefotaxime, Ofloxacin, Gentamycin, Amikacin, Lomefloxacin, Ciprofloxacin and Cefaperazone. They were, however resistant to ampicillin, Netilline and Cefuroxime and Pefloxacin and hence these must not form part of the empirical antibiotics for the treatment of *Proteus* infections at MGM Hospital. 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 .

Acknowledgement

We will like to acknowledge the Medical Microbiology Laboratory technicians of MGM HOSPITAL for their assistance in this study.

References

Barrow, G.I., and Felthan, R.K.A. 2003. Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd Ed. Cambridge University Press. Cambridge UK. 351-353.

Cheesebrough, M., 2000. District Laboratory Practice in Tropical Countries. Part 2, Cambridge University Press, UK. 132–143.

Chow, A.W., et al. 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

Chung, H.I., et al. 1999. Prevalence of *Proteus* species in urinary tract infections in a regional hospital in Trinidad. 62:438-442.

Douglas, G., et al. 2000. Bennett's Principles and Practice of Infectious Diseases. 5th ed. Philadelphia, Pa: Churchill Livingstone. 9:121-126

Emori, G.T., et al. 1993. An overview of nosocomial infections, including the role of the microbiology laboratory, Clin. Microbiol. Rev. 6: 428.

Foxman, B., 2000. Urinary tract infections: Incidence and risk factors. Am. J. Pub. Health, 80:331-333.

Heinzelmann, M., et al. 2002. Factors predisposing to bacterial invasion and infection. Am J Surg; 183(2): 179-90

Jones, R., et al. 2003 Bacteraemia, England, Wales and Northern Ireland: Commun Dis Rep CDR. Wkly [serial online]

Levy. S.B., 1999. Antibiotic resistance: an ecological inbalance. Ciba Found. Symp. 207**:**1-14.

Mobley, H.L.T., 1994. Virulence of *Proteus mirabilis* in urinary tract infections. Molecular pathogenesis and clinical management. ASM Press, Washington, D.C. 245–269.

Nawal, V.I., et al. 1994. In vitro antimicrobial activities against *Proteus* species isolated from patients in a Tertiary care Hospital in Jordan: Frequency of isolation and Epidemiology. Scienti.Res.Essay. 3(4):122-126.

Newman, M.J., et al. 2006. Resistance to Antimicrbial Drugs in Ghana. The Ghanaian – Dutch collaboration for Health Research and Development, 1-6.

Ohene, A., 1997. Bacterial pathogens and their antimicrobial susceptibility in Kumasi, Ghana, East Afri. Med. J. 74:450–455.

Patterson, J.E., et al. 1999. Association of antibiotic utilization . Measurement and control of multiple drug resistance.19(8):120-128.

Reslinski, A., et al. 2005. Prevalence of multi-drug resistant *Proteus* species in clinical specimens and their susceptibility to antibiotics, Med. Dosw. Micribial, 57(2):175-184.

Tenssaie, Z.W., 2001. Multiple antimicrobial resistance in gram negative bacilli isolated from clinical specimens, Jimma Hospital, Southwest Ethiopia, Ethiopia Med. J. 39: 305 –312.

Yah, S.C., et al. 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.

Yao, J.D.C, et al., 1999. Antibacterial agents. Manual of clinical microbiology. 7th ed. Washington, D.C. American Society for Microbiology. 1474–1504.