African Journal of Biotechnology Vol. 8 (5), pp. 725-730, 6 March, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Incidence of *Proteus* species in wound infections and their sensitivity pattern in the University of Benin Teaching Hospital

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Accepted 9 December, 2008

Proteus **species are frequently recovered from infected wounds. They contaminate wounds and thus cause infections. This study was carried out at the University of Benin Teaching Hospital (UBTH) to determine the involvement of *Proteus* species as one of the major causative organisms in wound infections. The study also determined the sensitivity pattern of the *Proteus* isolates. This was a prospective and cross-sectional study. Wound swabs and aspirates from various parts of the body and consisting of accidental, pathological and post-operative wounds were collected from patients who attended the clinics at the UBTH and examined by standard bacteriological methods. All isolates were tested for sensitivity against ciprofloxacin 5 µg, gentamycin 10 µg, streptomycin 10 µg, ofloxacin 5 mg/µg, chloramohenicol 10 µg, erythromycin 10 µg and tetracycline 10 µg. Of the 400 wound samples from various parts of the body 390 (97.5%) yielded growths and produced 560 isolates. Ten samples (2.5%) yielded no growths. *Proteus* species accounted for 150 (26.8%) of the isolates. *Proteus mirabilis* was the *Proteus* species most commonly isolated, 97 (17.3%), *Proteus vulgaris* 40 (7.1%), *Proteus rettgeri* 8 (1.40%), and *Proteus morgagni* 5 (0.9%). All the isolates were sensitive to ciprofoxacin, ofloxacin and gentamycin while all were resistant to tetracycline and erythromycin. Knowledge of the microbial flora of an environment and the sensitivity pattern are important tools in the management of wound infections especially those caused by *Proteus* species, and are also useful in formulating rational antibiotic policy.**

Key words: Wounds, infections, *Proteus* species, antibiogram.

INTRODUCTION

A wound is the disruption in the continuity of soft parts of the body structures (Singleton and Sainsbury, 1978; Torpy et al., 2005). Development of wound infection depends on the interplay of many factors. The breaking of the host protective layer- the skin, and thus disturbing the protective functions of the layer, will induce many cell types into the wound to initiate host response (Collier, 2003) Infection of the wound is the successful invasion, and proliferation by one or more species of micro-organisms anywhere within the body's sterile tissues, sometimes resulting in pus formation (Calvin, 1998).

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Wounds can be classified as accidental, pathological or post-operative. Whatever the nature of the wound, infection is the attachment of microorganisms to host cells and they proliferate, colonize and become better placed to cause damage to the host tissues (Collier, 2003).

Wound can be infected by a variety of microorganisms ranging from bacteria to fungus and parasites (Bowler et al., 2001). The common gram-positive organisms are the β-hemolytic streptococcus – *Streptococcus pyogenes* and *Staphylococcus aureus*. The gram negative aerobic rods are *Pseudomonas aeruginosa*. The facultative anaerobes include enterobacter species, *Escherichia coli*, klebsiella species and *Proteus* species. The fungal organisms are Candida species and moulds (Aspergillus species) (Gus Gunzalez et al., 2006).

The *Proteus* group belongs to the family of Enterobac-

teriaceae. The tribe Protease includes three genera which are *Proteus*, *Morganella* and *Providencia* (O'Hara et al., 2000; Mckell and Jones 1976). The genus, *Proteus* is found in soil, water, and faecally contaminated materials. *Proteus* species show a characteristic swarming motility, which is observed, on non-inhibitory agar medium as a wave-like movement across the entire surface of agar medium. Whenever swarming is observed *Proteus* species should be suspected. *Proteus mirabilis* is the species most commonly recovered from humans, especially from urinary and wound infections. It accounts for 90% of all infections caused by the *Proteus* species (Auwaerter, 2008). It is however not involved in nosocomial infections as do the indole positive species (Auwaerter, 2008). *Proteus vulgaris* is most frequently recovered from immuno-compromised patients or those on long-term antibiotic regimen. *P. mirabilis* is indole negative, chloramphenicol and ampicillin sensitive while *P. vulgaris* is indole positive and chloramphenicol resistant (Hickman et al., 1982 Gus Gunzalez et al., 2006). *Proteus penneri* resembles *P. vulgaris* except in being indole negative and not producing hydrogen sulphide. It is however differentiated from *P. mirabilis* by being resistant to chloramphenicol (Hickman et al., 1982). The other two species of *Proteus*, *Proteus morganii* and *Proteus rettgeri*, do not produce hydrogen sulphide like *P. mirabilis* and *P. vulgaris*. *P. rettgeri* is citrate positive and does not form gas from glucose while *P. morganii* is citrate negative and forms gas from glucose. The *Proteus* species which are pathogens of human are *P. mirabilis*, *P. vulgaris*, *P. morganii* and *P. rettgeri.* As members of the enterobacteriaceae, they are all oxidase negative, actively motile, non spore forming, non capsulated and are recognized by their ability to cause disease (Auwaerter, 2008). Infection with *P. penneri* is limited to urine and wound in the abdomen, groin, neck and ankle and is rare in human (Krajden et al., 1987). These *Proteus* species are part of the gram negative bacilli implicated in wounds, especially in diabetic wounds along with *E. coli*, *Enterobacter* species, *Klebsiella* species and *Serretia marscens* (Goldstein et al., 1996; Mutta et al., 2003; Jun et al., 2006; Raviskhar et al., 2006; Bowler et al., 2001). *Proteus* species are found in long-term care facilities and hospitals (Beck-Sague and Giuliano, 1994; Gus Gonzalez et al., 2006). They cause significant clinical infections, which are difficult to eradicate especially from hosts with complicated wounds, catheterization and underlying diseases and the immuno-compromised (Mutta et al., 2003; Asad and Amna, 2004; Abdel et al., 2005; Ravisekhar et al., 2006; Gus Gonzalez et al., 2006; Auwaerter 2008). The *Proteus* species, which colonize the intestinal tract, differ from those found in wounds in their ability to carry genes encoding antibiotic resistance (Asad and Amna 2004; Yah et al., 2007). The routine use of antibiotics in both medical and veterinary medicine has resulted in wide spread antibiotic resistance and development of antibiotic resistance genes especially within the gram negative organisms (Sengupta et al., 2001; Singh et al., 2003; Enabulele et al., 2006).

As members of the genus *Proteus* occur widely in man, animals and in the environment and can be readily recovered from sewage, soil, garden vegetables and many other materials (Gus Gonzalez et al., 2006), it is necessary to control the organisms since the sources of infection are many. There are numerous reports in the literature on the incidence of *Proteus* species in wound infections. They include those of Umebese et al. (2004), Ravisekhar et al. (2006), Jun et al. (2006), Yah et al. (2007) and Auwaerter (2008). Despite the literature cited above no such work has been reported in the University Of Benin Teaching Hospital. As *Proteus* species are found in multiple environmental habitats including long-term care facilities and hospitals, and they cause significant clinical infections, it becomes necessary to have a continued surveillance of the organisms. This is important to alleviate morbidity and mortality rates due to these organisms in University of Benin Teaching Hospital. The aim of this work is to determine the bacteriological profile of wound infection and know the antibiotic susceptibility pattern of the *Proteus* species in University of Benin Teaching Hospital environment.

MATERIALS AND METHODS

Specimens

Four hundred wound specimens were processed between January 2006 and December 2007. Specimens were collected from patients attending the general practice clinics, the medical and surgical clinics of the UBTH, Benin City. The categories of wound infections involved in the study included burns, bruises, bedsores, trauma wounds, post operation sepsis, cellulitis, ulcers, abscesses and osteomyelitis.The UBTH is located in Benin City. It is a large tertiary referral centre, which serves the whole of Edo State and beyond. Benin is predominantly a civil servants town and has a few businessmen**.**

Cultural technique

The various media used included Blood agar (Oxoid No. Cm271), McConkey agar (Oxoid No. Cm7) Robinsons cooked meat (Oxoid Cm 81). Nutrient agar (Oxoid No. Cm2) was used for sensitivity. The agar plates were dried in the oven to remove moisture. Culture was by the streak method after Cheesbrough (2000). The plates were streaked aseptically with sterile wireloop to form discrete colonies. A grease-fire glass slide was smeared with the specimen for gram staining.

The streaked plates were then incubated at 37°C for 24 h before being observed for any possible growth. Plates were not discarded until after 48 h. For anaerobic incubation a sachet of hydrogen-gas generating kit, anaerobic system (Oxoid No. BR 388) was introduced into an anaerobic jar along with plates to be incubated. The sachet was cut off at the corner as indicated by broken lines and a measured 10 ml of water added into the sachet in order to produce hydrogen gas for the anaerobiosis. The anaerobic jar was incubated along with the aerobic plates for a maximum of 48 h. All isolates were identified according to the methods advocated by Cowan and Steel (1993). The specific methods involved were colonial characteristics on media including size, inability to swarm, ability or inability to ferment lactose. Specific tests such as oxidase, catalase, carbohydrate utilization tests, indole formation and citrate tests were

Table 1. Distribution of isolates from different types of wound infections in UBTH.

Nature of wound infection	No. of samples	No. of samples yielding growth	No. of isolates	% of isolates
Pathological	160	160	250	44.644
Trauma	140	137	185	33.04
Post-operative	100	97	125	22.04
Total	400	390	560	100

done. Susceptibility test was done by the disc diffusion method, a modification of Bauer et al. method (1966). This was a function of the antibiotic initial concentration, its solubility and diffusion rate through agar. Commercially prepared antibiotics discs were placed on the nutrient agar plates that have been inoculated with the test organisms with sterile forceps at least 35 mm apart.

The different antimicrobial agents used and their disc contents were erythromycin 5 µg, tetracycline 10 µg, gentamycin 10 µg, chloramphenicol 10 µg, streptomycin 10 µg, ampicillin 10 µg, ofloxacin 10 µg and ciprofloxacin 5 µg. Plates were incubated for 24 h at 37°C after which the zone of inhibition was measured. Sensitivity on anaerobes was done on blood agar plates and incubated anaerobically. In reporting the results, resistance to any antibiotic was represented by R, while S represented sensitivity of the organism to the antibiotic. A standard sensitive strain of *Escherichia Coli* cw3310 was included as the control organism.

RESULTS

There were 400 swabs examined. 390 (97.5%) swabs yielded growth of 560 isolates. This means that some samples yielded more than one organism. Ten (2.5%) wound swabs failed to yield any growth. There was a distribution of isolates from the different types of wound infections (Table 1). Pathological wound infections were 160 samples with a total of 250 (44.64%) isolates; trauma wound swabs were 140 and yielded 185 (33.04%) isolates; post-operative wounds were 100 samples and yielded 125 (22.32%) isolates.

There was a distribution of *Proteus* species isolated from various wound infections. A total of 150 *Proteus* species (26.80%) were isolated. Seventy (12.5%) isolates were from pathological wounds; 50 (8.9%) *Proteus* isolates were from trauma, and 30(5.4%) from post operative wounds (Tables 2 and 3). Apart from the various *Proteus* species, other bacteria species were also isolated and their frequency of occurrence was demonstrated. *Staphylococcus aureus* was the most frequently isolated organism 168 (30%); Others were *Pseudomonas* species 23.6%, *E. coli* 11.6% *Klebsialla* species 37 (6.61%), *Streptococcus* species 5(0.8%); Providence species 3 (0.5%) and Enterobacter species 2 (0.36%) (Table 4).

Proteus species had the highest frequency of occurrence among the gram negative bacteria isolated. At species level *P. mirabilis* was the commonest followed by *P. vulgaris*, *P. rettgeri* and *P. morganii* in descending order (Table 5). There was a demonstration of *Proteus* species from the different wound infection sources and their antibiotic sensitivity pattern.

Table 2. Frequency of *Proteus* species from different wound infections in UBTH.

Nature of wound infections	No. of species isolated	% of isolates
Pathological	70	12.5
Trauma	50	8.9
Post-operative	30	5.4
Total	150	26.8

Table 3. Frequency of *Proteus* species isolated in UBTH.

Proteus sp.	No. isolated	% isolated
Proteus mirabilis	97	17.32
Proteus vulgaris	40	7.14
Proteus rettgeri	8	1.42
Proteus morgani	5	0.89
Total	150	26.8

DISCUSSION

This study clearly showed that *Proteus* species were the most commonly isolated organisms amongst the Gram negative facultative anaerobic bacilli. This is however contrary to the observations of Kehinde et al. (2004) who claimed that *Klebsiella* species were most predominant in burn wounds at 34.4% followed by *Pseudomonas* species (29%) and *Proteus* species the least prevalent (6.5%). Again Ihanni et al. (2003) and Oni et al. (1997) working in a tertiary hospital in Ibadan observed different prevalence rates of the gram negative organisms in their work in which *Proteus* species were the least prevalent. The difference in the *Proteus* species prevalence rates may be related to their distribution in the various environments. However Oguachuba (1985) reported that *Proteus* species were the commonest coliforms isolated from wound infections. Of the four pathogenic species of *Proteus*, *P. mirabilis* was the most common, followed by *P. vulgaris. P. rettgeri*, and *P. morgani* in decreasing order. Although *P. mirabilis* was isolated more frequently than the other *Proteus* species in this study (64.5%). it is less than 90%, as claimed by Auwaerter (2008). The same trend was observed by Sapica et al. (1980), Lambe and Ferguson (1991), and Wheat et al. (1996). However

Table 4. Frequency of isolates from various wound infections in UBTH.

Organisms	Single isolated	Mixed isolates	Total no. of isolates	% isolated
S. aureus	80	88	168	30
Proteus species	70	80	150	26.8
Pseudomonas sp.	54	76	130	23.61
Escherichia coli	30	35	65	11.61
Klebsialla sp.	10	25	35	6.25
Strptococcus sp.	-	5	5	0.9
Providence sp.	-	3	3	0.45
Enterobacter sp.	-	2	2	0.36
Total	244	316	560	100

the observations of Klainer and Bisaccia (1991) were contrary. While this study indicated that *P. mirabilis* was most prevalent, Sapica et al. (1980) failed to observe *P. mirabilis* among the *Proteus* species isolated from infected wound specimens. Klainer and Bisacca (1991) observed very few *Proteus* species and there was a complete absence of *P. mirabilis.* The *Proteus* species in their study had a near insignificant percentage to cause any clinical anxiety. Today *Proteus* organisms are known to cause significant clinical infections and occupy multiple environmental habitats (Gus Gonzalez et al., 2006). The varying pattern of microbial isolates among different studies emphasized the need for surveillance to evaluate and monitor periodically the changing pattern of the microflora in a hospital setting (Obunge et al., 2002).

Proteus species usually show high resistance to commonly used antibiotics (Steward and Beswick, 1977). In this study all the *Proteus* species isolated were sensitive to ofloxacin and ciprofloxacin of the fluorinated quinolone group and gentamycin an aminoglycoside. They indicated a therapeutic efficacy of close to 100% in this study. These antibiotics are therefore recommended as an effective single broad-spectrum antibiotic both in empirical and prophylactic treatment. In the literature, sensitivity to chloramphenicol was a way of differentiating *P. mirabilis* from *P vulgaris* in being sensitive to chloramphenicol. In this study it was observed that most of the *P. mirabilis* were resistant to chloramphenicol. This observation can be explained on the widespread plasmid resistance genes among *Proteus* species (Yah et al., 2007; Enabulele et al., 2006; Auwaerter, 2008; Jun et al., 2006). The possibility of observing more than one gram negative isolate in a wound facilitates the exchange of plasmid resistance genes between organisms.

This study encourages the use of the floroquinolones and gentamycin as the antibiotic of choice in wound infections as they continue to be effective and gave the widest cover as single antibiotics. This study also demonstrated the common bacteriological profile of infected wounds in this environment and gave a guide to antibiotic treatment of *Proteus* wound infections especially in situations where laboratory results are not readily available or completely lacking. In University of Benin Teaching Hospital chloramphenicol cannot be the drug of choice for the treatment of wound infections caused by *P. mirabilis* as this study has refuted the literature claim.

All the *Proteus* species in this study were resistant to erythromycin and tetracycline. The antibiotic susceptibility tests demonstrated that *Proteus* species have a wide range of resistance to several antibiotics. This could be a result of the extra outer cytoplasmic membrane which contains a lipid bilayer, lipoproteins, polysaccharides and lipopolysaccharides, and of course, abuse and misuse of antibiotics could be part of the contributing factors of resistance to antibiotics. It is advisable that treatment of *Proteus* wound infection be guided by the sensitivity result since the antibiotic susceptibility pattern of each species, depends on the extent to which the use of the various drugs has either selected resistant mutant or promoted the transfer of resistance factor from other members of the enterobacteriaceae (Yah et al., 2007).

Literature reports indicated that most strains of *Proteus* are susceptible to cotrimoxazole and almost all species are sensitive to gentamycin (Duguid et al., 1978). However, the *in vitro* sensitivity in this study did not show gentamycin to be the drug of choice for *Proteus* infections rather the quinolones, ciprofloxacin and oflxacin were observed to be the drugs of choice for the treatment of *Proteus* infections. .

This study showed the frequency occurrence of the various *Proteus* species observed in wound infections in UBTH and their susceptibility pattern. *P. mirabilis* was observed as the most frequently isolated in keeping with the observations of Auwaerter (2008). The study refuted some literature claims on the susceptibility pattern of *P. mirabilis* and advised that treatment of any *Proteus* wound infection must be guided by the laboratory sensitivity result as the species vary in their susceptibility pattern due to transfer of plasmid resistant genes among the gram negative organisms (Enabulele et al., 2006; Yah et al., 2007; Auwaerter 2008). It should be remembered that every apparently healthy person is capable of infecting himself and other people as *Proteus* species are ubiquitous in the environment; therefore good personal and environmental hygiene are necessary. There is need to maintain proper hygiene standards within hospital sur-

Table 5. Drug sensitivity (%) pattern of *Proteus* species from wound infections in UBTH.

Proteus	Ciprofloxacin (5 µg)	Gentamycin (10 µg)	Ofloxacin (5 µg)	Chloramphenicol (10 µg)	Erthromycin (10 µg)	Tetracycline (10 µg)	Streptomycin (10 µg)	Ampicillin (10 µg)
P. mirabilis	100	85	100	28	0	0	30	20
P. vulgaris	100	85	100	23	0	0	28	11
P. rettgeri	100	80	100	0	0	0	0	10
P. morgani	100	83	100	0	0	0	0	0

roundings to reduce the incidence of infections especially nosocomial infections as *Proteus* species (except *P. mirabilis*) can be incriminated in nosocomial infections (Auwaerter, 2008). Future studies on *Proteus* species should be encouraged because some more new grounds as regards distribution and susceptibility to anti-microbial agents may be discovered. There is need for hospitals to encourage an annual review of the microbial flora of their environment and the antibiotic sensitivity pattern. This study encourages the use of the floroquinolones and gentamycin as the antibiotic of choice in *Proteus* wound infections. This study also demonstrated the common bacteriological profile of infected wounds in this environment and provided a guide to antibiotic treatment particularly those wound infections that are caused by the various species of *Proteus*.

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