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Prevalence and antibiotic resistance patterns of *Proteus mirabilis* isolated from catheter-associated urinary tract infection.

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ABSTRACT

The aim of this study was to determine the frequency of the bacterial agents that cause urinary tract infection in catheterized patients at Zagazig University Hospital and to assess the antimicrobial resistance pattern of *Proteus mirabilis* isolates against various antimicrobial agents used for treating urinary tract infections in the spate of its recorded increasing resistance patterns.Urine specimens were obtained from catherized patients suffering from a urinary tract infection (UTI). The different isolates obtained were identified by conventional microbiological methods.The antibiotic susceptibility profile of *Proteus mirabilis* isolates was determined by agar dilution method. Patient's medical records showed significant increase in incidence of catheter associated urinary tract infection (CA-UTI) upon prolonged catheterization, among immunocompromised patients and patients with concomitant chronic diseases, elderly patients and female gender.

The frequency of the obtained isolates was *Eschersheia coli* (33%), *Klebsiella pneumoniae* (14%), *Enterococcus faecalis (13%), Proteus species* (10.2%), *Pseudomonas aeruginosa* (10%), *Staphylococcus aureus* (10 %) and unidentified gram positive cocci (9.8%). 90% of the *Proteus* isolates were *Proteus mirabilis*. The resistance of *P. mirabilis* isolates were highest against Nalidixic acid, Ampicillin/Sulbactam, Amoxicillin/clavulanic acid, ceftazidime and cefuroxime (95.5%-57.8%). Lower resistance rates of *P. mirabilis* were found against amikacin, levoflxacin, ciporofloxacin and gentamicin (2.2 % -17.8 %). While *P.mirabilis* isolates were 100% sensitive to Imipenem. In conclusion, Imipenem is recommended to be used as an empirical treatment of CA-UTI caused by *P. mirabilis*.

INTRODUCTION

Urinary tract infections are the most common infections after upper respiratory tract infections (Hryniewicz *et al*., 2001) and they are the most prevalent type of nosocomial infections accounting for 25% to 40% of nosocomial infections (Bagshaw and Laupland, 2006). Catheter associated urinary tract infections (CA-UTI) are the most common type of nosocomial infections, accounting for 80% of all nosocomial UTIs (Hartstein , 1981). Bacteria are the major causative organisms for UTIs and are responsible for more than 95% of UTI cases (Bonadio *et al.*, 2001). *Proteus mirabilis* is the third most common cause of complicated UTI (12%) and the second most common cause of catheter-associated bacteriuria in long term catheterized patients (15%) (Warren, 1996). It was observed that *P. mirabilis* has the greatest ability to attach to catheters out of all gram-negative organisms (Roberts *et al*.,1990). The ability of *P. mirabilis* to colonize the surfaces of catheters and urinary tract is believed to be aided by a characteristic known as swarming differentiation and migration (Allison *et al.*,1994). Furthermore, the expression of other virulence factors of *Proteus* including urease and protease is coordinately upregulated during swarming (Rather, 2005). Antimicrobial resistance offered by different uropathogens is one of the barricades that might hinder a

successful treatment as antimicrobial resistance pattern varies with time (Dyer,1998). Lack of appropriate laboratory facilities to carry out comprehensive antimicrobial sensitivity patterns leads to wrong empirical drug selections with attendant treatment failures and propagation of resistant bacteria (Noreddin *et al*., 2011).

This study was set up to investigate the prevalence and antibiotic susceptibility patterns of uropathogenic *Proteus* isolated from catheterized patients at Zagazig University Hospital so as to offer a guide to clinicians for proper antibiotic selection for empirical treatment.

MATERIALS AND METHODS

Media and chemicals

MacConkey agar, Cysteine lactose electrolyte deficient agar (CLED), Nutrient agar, Mannitol salt agar, Eosin-methylene blue (EMB) agar, Mueller Hinton agar were obtained in dehydrated form from Oxoid, Hampshire, England (Hampshire, UK). Triple sugar iron (TSI) agar, Simmon's citrate agar and Bile esculin agar was purchased from Lab M Limited (Lancashire, United Kingdom.). The other culture media were prepared from their components according to Koneman *et al*. (1997).

Antimicrobial agents were of pharmaceutical grades. A total of thirteen antimicrobial agents were used in the study.Azetronam was obtained from Bristol-Myers Squib, Egypt. Amoxicillin /clavulinic acid were obtained from Sedico pharmaceutical company,Egypt. Ceftriaxone, cefuroxime and imipenem was obtained from Glaxo Smith Kline, Egypt. Ampicillin/sulbactam and cefoperazone was obtained from Pfizer, Egypt. Ciprofloxacin, levofloxacin, cefotaxime, amikacin and gentamicin were obtained from Egyptian Pharmaceutical Industries Company (EPICO), Egypt. Ceftazidime was obtained from Smith Kline Beecham, Egypt. Nalidixic was obtained from Memphis Chemical company, Egypt. Nitrofurantoin was obtained from Mepaco-Medifood, Egypt.

Isolation and idetification of the isolates

A total of 520 urine specimens were collected from catheterized patients with urinary tract infections comprising 203 males and 317 females aged between 4 years to 80 years were assessed between the period from October 2009 to September 2010. The samples were collected from patients admitted to Zagazig University Hospital. All patients have been catheterized minimum for 4 days and maximum for 30 days. All specimens were aseptically collected and transported immediately to the microbiological laboratory, where they were processed. The specimens were cultured on Blood agar, CLED agar using 1 µl calibrated loop and incubated aerobically at 37°C for 24 h to detect colony forming unit, count more than 10^5 cfu/ml was considered significant. The isolates were identified by morphological, Gram stain and biochemical reactions according to Koneman *et al*. (1997).

Determination of minimum inhibitory concentration (MIC) by the agar dilution method

Minimum inhibitory concentrations (MIC µg/ml) of the selected antimicrobial agents against *Proteus mirabilis* isolates were determined by agar dilution method according to Clinical Laboratory and Standards Institute (CLSI, 2010).

Preparation of the inoculum

From a pure culture, at least 3-4 colonies were picked. Resolve totally in 4-5ml saline, mix and adjust

turbidity to 0.5 McFarland standard. The bacterial suspension then diluted 10-fold to yield the final inoculums suspension of 10^7 CFU/ml. The bacterial suspension should be used for inoculation within 15 minutes.

Preparation of agar dilution plates

The appropriate dilutions of antimicrobial solutions were prepared and added to molten Mueller-Hinton agar (3% agar to minimize the swarming) at 45 to 50°C, mixed and poured into petri dishes.

Inoculating agar plates

A standardized micropipette was used to deliver 1µl of the suspension to the surface of Mueller-Hinton agar containing antibiotic dilutions and the control plates (without the addition of the antimicrobial agent), so that the final inoculum on the agar contains approximately 10^4 cfu/spot. The inoculated agar plates were allowed to stand at room temperature until the liquid was absorbed into the agar. The plates were inverted and incubated at 35-37°C for 16–20 hours, and examined to determine the MIC. The results were recorded and interpreted according to CLSI (2010) as susceptible (S), intermediate (I) and resistant (R).

STATISTICAL ANALYSIS

Statistical package for social sciences (SPSS; version 10; USA) was used to analyze data. Comparison of categorical variables and percentages between groups was done by one-sample *T test*, one-way ANOVA, as appropriate. A probability of $P<0.05$ was considered significant.

RESULTS

Patient's medical records were reviewed to identify the duration of catheterization and other factors that may contribute to the development of catheter associated urinary tract infection (CA-UTI). Figure (1) showed significant increase in the incidence of catheter associated urinary tract infection upon prolonged catheterization. In addition, patients' medical records (data not presented) cleared that 82% of the patients were immunocompromised and had concomitant chronic diseases such as diabetes, renal dysfunction, cancer, cardiovascular events and neurologic disorders (data not presented). Table (1) revealed the percentages of occurrence of CA-UTIs among the different age/gender groups. In females the highest percentage (52.7%) of CA-UTI cases was observed in patients who are aged ≥ 60 years, followed by (14.2%) in patients aged 50-59 years. However, the highest percentage (42.9%) of CA-UTI in males was observed in patients who are aged ≥ 60 years, followed by (15.7%) among patients aged 40-49 years. Furthermore, 9.6% of CA-UTI were children under 10 years. While there was no significant difference between males and females at the age group 4-9 years ($P > 0.05$). In addition, our results suggested that the occurrence of CA-UTIs in females is significantly higher than that of male ($P<0.05$). There was significant difference between the total number of male and female patients in the incidence of occurrence of CA-UTIs (Table 1 & Figure 2).

Out of 520 urine specimens collected from catheterized patients with urinary tract infections in Zagazig University Hospital from Department of Urology and Intensive Care Units from October 2009 to September 2010. 490 specimens (94%) gave positive bacterial growth with a viable count $>10^5$cfu/ml. The isolates recovered were identified and results were presented in table (2). *Escherichia coli* was the most frequently isolated pathogen (33%), followed by *Klebsiella pneumonia* (14%),

Enterococcus faecalis (13%), *Proteus* spp (10.2%), *Pseudomonas aeruginosa* (10%), *Staphylococcus aureus* (10%) and unidentified Gram positive cocci (9.8%). In addition, out of the fifty *Proteus* isolates, 45 were identified as *Proteus mirabilis* (90%), while 5 were *Proteus penneri* (10%).

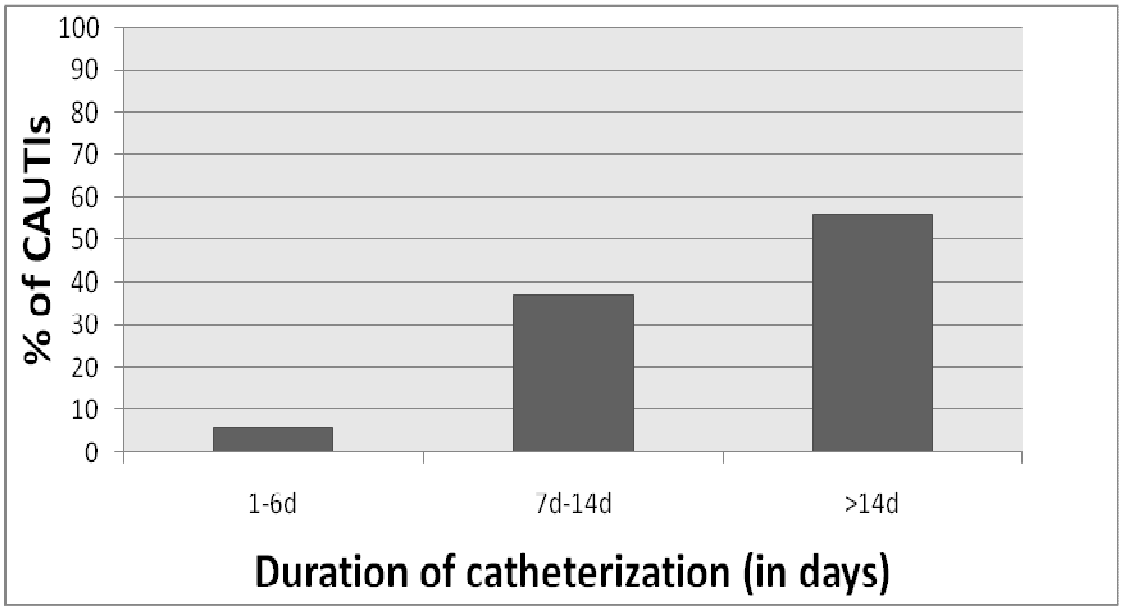

Figure 1. Correlation between duration of catheterization and occurrence of Catheter associated urinary tract infections.

Table 1. Age and gender distribution of catheter associated UTIs.

Age (years)	Male		Female		Total	
	No.	%	No.	%	No.	%
4 -9	20	9.8	30	9.5	50	9.6
10 -19	15	7.4	20*	6.3	35	6.7
20 -29	12	6	14*	4.4	26	5
30 -39	11	5.4	20*	6.3	31	6
40 – 49	32	15.7	21*	6.6	53	10.2
50 – 59	26	12.8	45*	14.2	71	13.7
≥60	87	42.9	167*	52.7	254	48.8
Total	203	39	317*	61	520	100

* Significantly higher ($P < 0.05$) by one-sample *T test*

Table 2. Frequency of the isolated pathogens from CA-UTIs.

Bacterial isolate	No.	%
Escherichia coli	162	33
Klebsiella pneumonia	68	14
Enterococcus faecalis	64	13
Proteus spp	50	10.2
Pseudomonas aeruginosa	49	10
Staphylococcus aureus	49	10
Unidentified Gram positive cocci	48	9.8

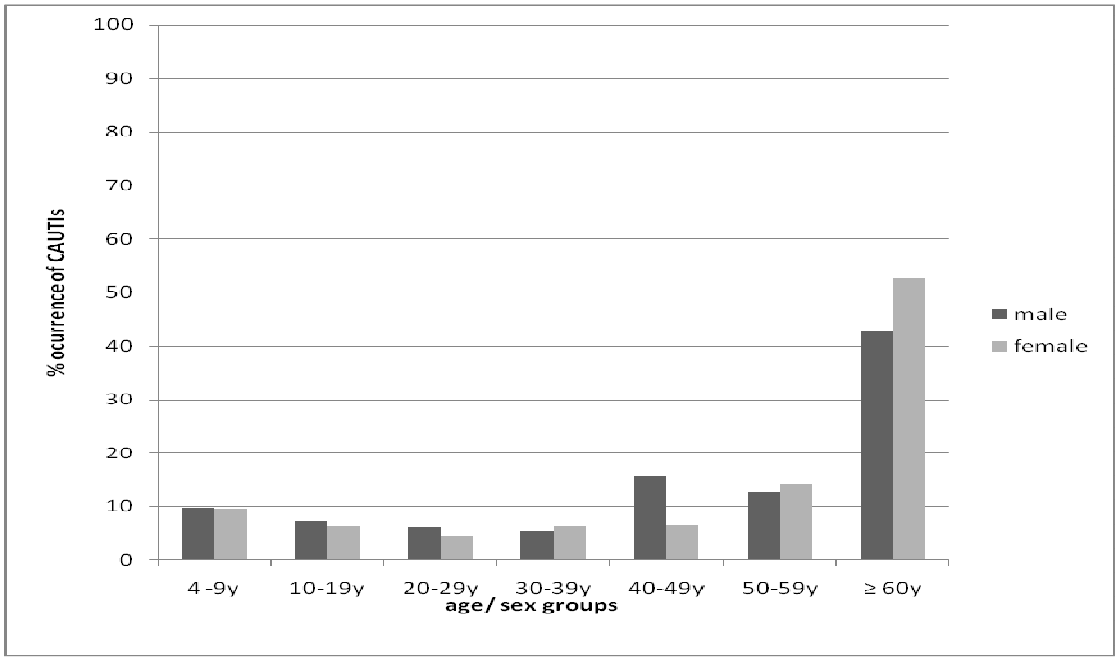

Figure 2. Correlation between age/gender and occurrence of CA-UTIs.

Minimum Inhibitory Concentration (MIC) of antimicrobial agents

The percentage of resistance of *Proteus mirabilis* to different antimicrobial agents used is presented in (Table 3). MIC_{50} and MIC_{90} of various antimicrobial chemotherapeutic agents *against Proteus mirabilis* isolated from the indwelling catheters are presented in (Table 4) in terms of MIC_{50} (MIC at which 50% of the isolates tested were inhibited), MIC_{90} (MIC at which 90% of the isolates tested were inhibited), and the range of MICs

Table 3. The percentage of resistance of *P.mirabilis* against various antimicrobial chemotherapeutic agents

Antimicrobial agent	Resistance rates of *P.mirabilis*
Imipenem	0 %
Amikacin	2.2 %
Gentamicin	17.8 %
Aztreonam	33.3 %
Cefotaxime	40 %
Cetazidime	60 %
Cefuroxime	57.8 %
Ciprofloxacin	8.8 %
Levofloxacin	4.5 %
Amoxicillin/Clavulinic acid	64.4 %
Ampicillin/Salbactam	75.5 %
Nalidixic	95.5 %
Nitrofurantoin	48.8 %

Table 4. MIC_{50} and MIC_{90} of various antimicrobial chemotherapeutic agents' against *P. mirabilis*

Antimicrobial agent	Range(µg/ml)	MIC(µg/ml)	
		MIC_{50}	MIC_{90}
Imipenem	0.5-4	1	4
Amikacin	4-32	8	16
Gentamicin	2-32	4	8
Aztreonam	2-32	2	32
Cefotaxime	4-64	4	64
Cetazidime	4-256	16	128
Cefuroxime	2-256	16	128
Ciprofloxacin	0.125-8	0.25	2
Levofloxacin	0.25-4	0.5	2
Amoxicillin/Clavulinic acid	4/2-128/64	32/16	128/64
Ampicillin/Salbactam	8/4-128/64	64/32	64/32
Nalidixic	1-128	8	64
Nitrofurantoin	32-512	128	256

A review of the antimicrobial susceptibility profile of P .*mirabilis* isolated from the clinical urine specimens form catheterized patients showed that Imipenem, Amikacin, Levofloxacin, Ciprofloxacin, Gentamicin and Azetronam were the most active. All *P. mirabilis* isolates were sensitive to Imipenem with MIC_{50} and MIC_{90} of 1 µg/ml, 4 µg/ml, respectively. Also, Aminoglycosides were highly active on *P. mirabilis*. Amikacin showed greater activity than Gentamicin as the resistance against Amikacin was 2.2%. On the other hand, resistance against Gentamicin was 17.8%. Furthermore, Quinolones (Levofloxacin and Ciprofloxacin) showed high activity on *P. mirabilis* isolates. Levofloxacin had an $MIC_{50\ and}$ MIC_{90} of 0.5µg/ml, 2µg/ml respectively, while Ciprofloxacin had an $MIC_{50\ and}$ MIC_{90} 0.25µg/ml, 2µg/ml respectively. *P. mirabilis* isolates showed 33.3% resistance to Aztreonam which is representative to monbactams. Nitrofurantoin was active on about half the *P. mirabilis* isolates, as the resistance rate against it was 48.8%. Cephalosporins as cefuroxime (second generation) had a resistance rate of 57.8%, while third generation cephalosporins as ceftazidime and cefotaxime showed 60%, 40% resistance rate respectively. High resistance rate were seen against ampicillin/sulbactam, amoxicillin/clavulinic acid. Also, Nalidixic acid showed 95.5% resistance rate.

DISCUSSION

Urinary tract infection (UTI) remains a worldwide therapeutic problem. Early diagnosis and prompt antimicrobial treatment are required to minimize its complications. The risk of developing a urinary tract infection increases significantly with the use of indwelling catheters .CA-UTIs can lead to many complications as cystitis, pyelonephritis, gram-negative bacteremia, prostatitis, epididymitis, and orchitis in males and, less commonly, endocarditis, vertebral osteomyelitis, septic arthritis, endophthalmitis, and meningitis in all patients (Foxman, 2003).

In the present study done in Zagazig university hospital, *Escherichia coli* (33%) was the most prevalent cause of CA-UTIs followed by *Klebsiella pneumonia* (14%), *Enterococcus faecalis* (13%), *Proteus* spp (10.2%), *Pseudomonas aeruginosa* (10%), *Staphylococcus aureus* (10%)

and Coagulase negative Staphylococci (9.8%). In a study by Warren (1996), *Proteus mirabilis* was the second most common cause of catheter associated bacteriemia in a group of long term catheterized patients**.**

Proteus mirabilis was found to be the most common *Proteus* species isolated from UTIs (Orrett, 1999). Other *Proteus* spp were found to be implicated in UTIs as Proteus *vulgaris* and *Proteus penneri* (Penner, 1992). This is similar to the results reported in our study as 90% out of the fifty *Proteus* isolates were *Proteus mirabilis* and 10% were *Proteus penneri*.

The results show that the number of CA-UI is higher with long catheterization. This is confident with results of other study by Hass (1982), who found that the risk of getting a CA-UTI increases at 5% per day of indwell time. Older age shows higher occurrence of CA-UTIs .In current study in both sexes the incidence of CA-UTIs was the highest in patient over 60 years**.** Senior (1987) reported that urinary tract infections with *Proteus*, were frequently only in patients of 60 years and above. Geriatrics may show higher incidence due to immuno-comprimisation. The frequency of UTI is greater in women 61% as compared to men 39% similar to results that were shown by Kashef *et al*. (2010).This could be owing to difference in anatomic and physical factors between genders**.**

Antimicrobial resistance profile of *Proteus* isolates revealed high resistance rates to six of the most frequently used antibiotics. *P. mirabilis* showed resistance rates of 95.5% to Nalidixic acid, 75.5% to Ampicillin/Sulbactam, 64.4% to Amoxicillin/Clavulinic acid, 6o% to Ceftazidime, 57.8% to Cefuroxime and 48.8% to Nitrofurantoin.

In the current study, *P. mirabilis* showed 48.8% resistance to nitrofurnatoin. In a study conducted in hospitals in Qassim province at Saudi Arabia the resistance rates of *Proteus spp*. to nitrofurantion was 81% (Alzohairy & Khadri, 2011). Therefore, the current study revealed that resistance of *P. mirabilis* to Nitrofurantin has increased greatly than that which was previously reported in a university hospital in Nigeria where the resistence rate was about 25% (Jombo *et al.*, 2012).

Moreover, resistance rates of *P. mirabilis* to Amoxicillin/ Clavulainic acid was 64.4% which is higher than that reported by Stratchounski *et al*. (1998), as they showed that the resistance rate was 20%. In another study in Nigeria by Jombo *et al* (2012) the resistance of *P .mirabilis* isolates was 62%**.** Consequently, it is apparent that the resistance rates are increasing with time.

The resistance rates of *P. mirabilis* to the different cephalosporins used in this study were 60 % to Ceftazidime, 57.8% to Cefuroxime and 40% to Cefotaxime. The current study reflects higher resistance profiles than previously reported by Stratchounski *et al*. (1998) who showed that the resistance rates of Proteus isolates to ceftazidime , cefotaxime were 1% , 20% respectively.

The resistance profile of the *P.* mirabilis to Nalidixic acid was much higher than that reported in a university hospital in Nigeria where the resistance rate was about 25% (Jombo et al., 2012).

The decrease in the susceptibility rates of *Proteus* isolates in the current study could be attributed to the overuse of nitrofurantoin, nalidixic, penicillins and cephalosporins in the hospital form which these isolates were collected, and to the misuse of these antibiotics.

These antibiotics are the most used as empiric treat without performing an antibiotic sensitivity test.

The sensitivity pattern for *Proteus* in this study shows that Imipenem was the most effective drug for *Proteus* induced urinary tract infection followed by Amikacin. This is in agreement with the results of Savas *et al*. (2006) who found that the most effective antibiotics against Gram-negative bacteria were imipenem and meropenem.While the resistance rate of *P. mirabilis* to amikacin was 2.2%.

In addition the current study showed that the resistance rate of *P. mi*rabilis to fluroquinolones is higher than the study of Shigemura, (2009) who reported 100% effectiveness of Ciprofloxacin. The widespread of flouroquinolones as ciprofloxacin and levofloxacin may have accelerated the development of resistance to these agents (Livermore *et al*., 2002).

In conclusion, the present study had shown that *Proteus mirabilis* isolates from urinary tract infections were highly resistant to most of the antibiotics in common use. Prudent and judicious use of antibiotics among physicians should be emphasized while control and spread of nosocomial infections should be seriously checked to limit the spread of resistant bacteria. Furthermore, Imipenem and Amikacin may be considered for empirical treatment of *Proteus mirabilis* in CA-UTIs if susceptibility reports are not readily available**.**

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تعين مدي انتشار و المقاومة للمضادات الحيوية لميكروب البروتيس ميرابيليس المعزول من عدوي المساك البولية الناتجة عن القسطرة المنزرعة في الحالب

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تهدف الدراسة الحالية لمعرفة المسببات البكتيرية لعدوي المسالك البولية الناتجة عن القساطر المنزرعة في الحالب في مستشفيات جامعة الزقازيق ومعرفة مدي مقاومتها للمضادات الحيوية الاكثر استخداما. تم جمع ٥٢٠ عينة بول من مرضى مصابين بعدوى المسالك البولية من مستخدمي القسطرة المنزرعة بالحالب وتم التعرف علي المسببات البكتيرية للعدوي ، وقد تم عزل (٤٩٠) عزلة تقسم إلى ٣٣% من العصويات المعدية (الايشيريشيا كولاي) وهي تمثل أعلى نسبة، ١٤% من جنس الكليبسيلا،١٣% من الانتيروكوكس فيكاليس ، ١٠.٢% من جنس البروتيس، ١٠% من السدومونس، ١٠% من المكورات العنقودية الذهبية (الإستافيلو كوكس أوريس) و ٩.٨% من المكورات العنقودية غير المعرفة. بالتعرف علي جنس البروتيس المعزولة وجد أن ٤٥ عينة هي بروتيس ميرابيلس و ٥ عينات هي بروتيس بينيري. ومقاومة البروتيس ميرابيلس كانت أعلي ما يمكن لحمض النليدسيك ، الامبيسلين/ سالباكتام، الاموكسيسيلين/حمض الكالفيولينك، السيفتازيديم والسيفيبوروكسيم بنسبة (٩٥.٥%- ٥٧.٨%) .أما نسبة المقاومة للاميكاسين، الليفيوفلوكساسين ، السيبروفلوكاسين والجناميسين فكانت أقل من المجموعة السابقة بنسبة مقاومة (١٧.٨ % - ٢.٢ %).وكانت جميع عزلات البروتيس ميرابيليس لها حساسية ١٠٠ % للاميبينيم.

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