The Isolation and Characterization of *Proteus mirabilis* from Different Clinical Samples

عزل وتشخيص بكتريا *Proteus mirabilis* المعزوله من عينات سريريه مختلفه

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Abstract

A total of one hundred five samples were collected from hospitals of Baghdad city during the period from 10/12/2008 to 15/3/2009. These clinical samples included: urine (50) wound swabs (25), sputum (20), and ear swabs (10). These samples were collected from the Baghdad hospital/ Teaching Laboratories, and Al-Yarmook Hospital/ Teaching Laboratories. Twenty six isolates of *Proteus mirabilis* were characterized according to the morphology and microscopic characteristics, along with the biochemical and confirmatory APi 20 E tests. These isolates were obtained from: urine (19), wound swabs (6), ear swabs (2), and sputum (1). The twenty selected isolates were tested for resistance against ten antibiotics and only urine samples were tested for nalidixic acid and nitrofurantoin resistance. It was shown that there were differences in the antibiotic resistance of isolates. High resistance to nitrofurantoin and ampicillin were found among isolates as (100%) and (75%) respectively while the resistance of *Proteu*s isolates to trimethoprin /sulphamethazol, were (65%). This study also showed that resistance of isolates to gentamicin, ciprofloxacin, ceftazidime, pipracillin, cefotaxime, nalidixic acid azteronam, imipenem and amikacin were (50, 40, 40, 40, 35,27, 20,15, 5)% respectively.

المستخلص

جمعت 105 عينة من مستشفيات مدينة بغداد (مدينة الطب/ المختبرات التعليمية ، مستشفى اليرموك / المختبرات التعليمية) إذ توزعت هذه العينات بواقع عينة إدرار (50)و (25) عينة من الجروح و (20) عينة قشع و (10) عينة من حالات التهاب الاذن. تم تشخيص 26 عزلة تعود إلى *Proteus mirabilis* اعتماداً على دراسة الخصائص المظهرية و المجهرية و ألاختبارات الكيموحيوية فضلاعن الاختبارات التأكيدية باستخدام نظام API 20E ، وقد كان توزيع تلك العزلات بين العينات كما يأتي: (19) عزلة تعود لعينات ألإدرار (6) عزلات تعود لعينات الجروح وعزلتين تعود لعينات القشع وعينه واحده تعودلعينات التهاب ألأذن . تم إجراء اختبار الحساسية الدوائية لـ (20) عزله اتجاه(10) مضادات حيوية و استخدم مضاد النايتروفورانتيون ومضاد حامض النالدكسك فقط للعزلات التي تعود لعينات ألإدرار، وقد أظهرت النتائج بان أعلى نسبة مقاومة كانت تجاه كل من مضاد النايتروفورانتيون 100% تبعه مضاد الامبسيلين 75 % ثم مضاد ترايميثوبريم \سلفاميثازول 65% . أما بالنسبة لباقي المضادات فقد تفاوتت النسب المئوية للمقاومة وكما يلي: الجنتامايسين50%، السبروفلوكساسين والسفتازديم وببراسيلين 40% ، السيفوتاكسيم 35% حامض النالدكسك 27% ألازتريونم 20% ، الاميبنيم15% وأخيرا الاميكاسين5%.

Introduction

The genus *Proteus* belongs to the tribe of Proteeae in the family of Enterobacteriaceae, this tribe consists of three genera: *Proteus, Providencia* and *Morganella*. These bacteria are gram negative rod measuring (1–3) µm in length and (0.4–0.8) µm in diameter, motile by

Key words: Isolation, Characterization, *Proteus*

peritrichous flagella, facultative anaerobic non spore forming, non capsulated, most isolates have fimbriae [1].

The genus *Proteus* consists of four species: *P.mirabilis*, *P. vulgaris*, *P. penneri*, and *P. myxofaciens*. The last of these is insignificant in infections of humans and has been isolated from living and dead larvae of the gypsy moth [1].

Proteus spp are the causative agent of a variety of opportunistic nosocomial infections including those of the respiratory tract, ear, nose, skin, burns, and wounds, it may also cause gastroenteritis [2].

Proteus species (*P. mirabilis, P. vulgaris*, and *P. penneri*) are important pathogens of the urinary tract and primary infectious agent in patients with indwelling urinary catheters [2]. Individuals suffering from urinary tract infections caused by *Proteus mirabilis* often develop bacteriuria, cystitis, kidney and bladder stones, and catheter obstruction due to stone encrustation, and acute pyelonephritis [3]. For the importance of *Proteus* spp. as a nosocomial pathogen, the present study was planned to perform the isolation of *Proteus spp*. from different sources and determination of antibiotic sensitivity of the selected (20 isolates).

Materials and Methods

Sample Collection

Total of 105 samples were collected from patients attending (Baghdad hospital/ Teaching Laboratories, and Al-Yarmook Hospital/ Teaching Laboratories. Samples were of wounds swabs, urine, sputum and ear swabs .The specimens were directly streaked onto macconkey and blood agar sand were incubated at 37ºC for 24 hours.

Identification of the Isolates

Isolates were identified depending on morphological and biochemical tests as compared with identification scheme described by [4], and according to API 20E confirmatory test**.**

Antibiotic Sensitivity Test (Qualitative Disk Method)

Twelve antibiotic disks(nalidixic acid, nitrofurantoin, ampicillin, trimethoprin /sulphamethazol, gentamicin, ciprofloxacin, ceftazidime, pipracillin, cefotaxime, nalidixic acid azteronam, imipenem and amikacin) were used to detect the sensitivity of 20 isolates of *P. mirabilis* according to method described earlier [5] .

Results and Discussion

Isolation and Characterization of *Proteus spp.*

One hundred five samples were collected from patients in Baghdad city hospitals. These samples were distributed as follows: urine (50), wound swabs (25), sputum (20), and ear swabs (10) as illustrated in table (1). Thirty local isolates were characterized depending on cultural and microscopic characteristic. Genus and species were characterized by using biochemical tests and API 20 E test as confirmatory test.

Table (1): Isolation source and percentage of *Proteus* spp. isolates

Isolation Source	Numberof Samples	Number of *Proteus* Isolates	Percentage of Total &Specific Isolation (1)Total Isolation	(2) Specific Isolation	(3) Percentage of Isolation Source%
Urine	50	20	19.04	66.6	40
Wound	25	6	5.71	20	24
Ear	10	2	1.90	6.67	20
Sputum	20	2	1.90	6.67	10
Total	105	30			

(1) The percentage of total samples (105).

(2) The percentage of total *Proteus* isolates (30).

(3) The percentage of each isolation source.

Cultural Characteristics

The *Proteus* isolates were firstly identified as related to the genus *Proteus* by swarming phenomenon on blood agar and the bacteria on the macconkey agar appeared pale [6]

Microscopical Characteristics

Microscopic examination of the bacteria appeared as straight rods and gram negative when it stained with gram stain [4].

Biochemical Characteristics

Several biochemical tests were done to characterize *Proteus* isolates. All the 26 isolates of *Proteus mirabilis* showed positive results to the biochemical tests, phenylalanine-deaminase, urease and KIA, but all were oxidase of citrate utilization test, negative. These isolates were motile, and all the 26 isolates were indole negative. Also *Proteus* isolates were unable to ferment lactose and maltose as illustrated in table (2) [4].

Table (2): Biochemical and motility tests of the *P. mirabilis* an isolates

Biochemical Test	*P.mirabilis*
Oxidase	–
Phenyl alanine Deaminase	+
Indole Production	–
Motility	+
Citrate Utilization	–
Urease Production	+
Gelatin Liquefaction	+
KIA	K/A*
lactose Fermentation	–
Maltose Fermentation	–

(-) a negative result, (+) a positive result. * K/A = ferments glucose with H2S gas production

For confirmation of the biochemical results, the API 20E strips were used for Enterobacteriaceae identification containing 12 tests [7]. The results revealed that the tested isolate were *P.mirabilis* as indicated in figure (1).

Figure (1): API 20 E confirmatory test for *P. mirabilis* characterization

Prevalence of *Proteus*

The result in table 1, observed that the percentage of *Proteus* spp. isolates among the total samples and isolates was (28.57%), and the percentage of *Proteus* spp. isolates according to the specimens was demonstrated in figure 2.

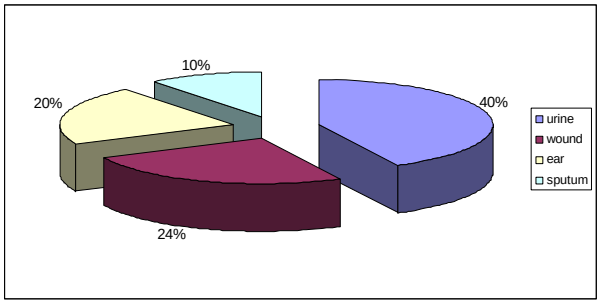

Figure (2): Percentage of *Proteus* spp from different specimens

As shown in the table (1), the percentage of *Proteus* spp which obtained from the urine specimens 40% which is a higher percentage compared with the other samples. In the study of [8] *Proteus* were isolated from urine, in a percentage of 56.9%.

The reason of the difference in isolate percentages may be due to the differences in size and number of hospitals surveyed as well as the season of collecting samples and medication taken before sampling. The bacteria has numerous virulence factors that are important for causing UTI and several of these factors appear to be more important for establishing infection in different areas of the urinary tract. These virulence factors include adherence capability, urease production and flagella [3].

The percentage of isolates obtained from wounds was 24%. *P.mirabili*s representing 20% of the wounds and burns among 79 different bacterial isolates and *Proteus* spp. had the highest frequency of occurrence among the gram negative bacteria isolated [9]. According to the reports of [10] *Proteus* spp. were found to be most dominant Gram-negative isolates in diabetic wounds. The lack of both presurgical prophylactic measures and education on the principles of asepsis for wound care may explain colonizing *Proteus* in wounds.

Proteus isolates percentage of ear infections was 20% as illustrated in table (1). The result is compatible with [11] who indicated that the isolation percentage of *Proteus* spp. was 26.9%, where as [12] found that the isolates percentage of otitis externa was 7.69% and *Proteus* spp. cause discharging ear which is clinical manifestation of otitis externa**.**

In sputum samples, there were 2 *Proteus* isolates from 20 samples and the isolates percentage was 10%, [13] indicated that the *Proteus* isolates percentage was 3.6%.

Antibiotic Resistance of *Proteus* isolates

Twenty selected isolates were tested for resistance toward ten antibiotics and only urine samples were tested for nalidixic acid and nitrofurantoin resistance. It was observed that there was a difference in the antibiotics resistance of isolates. High resistance to nitrofurantoin and ampicillin were found among isolates as (100%) and (75%) respectively, while the resistance of *P. mirabilis* isolates to trimethoprin /sulphamethazol were (65%). This study also observed resistance of *P. mirabilis* isolates to gentamicin, ciprofloxacin, ceftazidime, pipracillin, cefotaxime, nalidixic acid azteronam, imipenem and amikacin (50, 40, 40, 40, 35 ,27, 20 ,15 and 5) % consequently, as illustrated in figure 3.

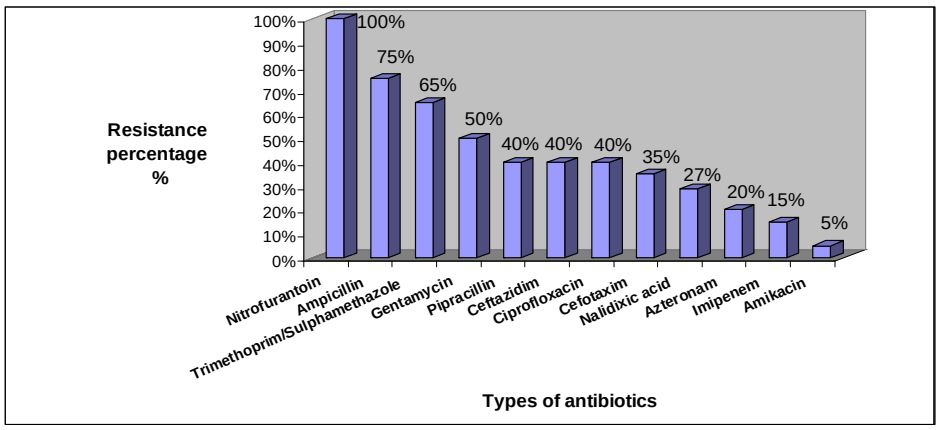

Figure (3) Susceptibility of *P.mirabilis* to Antibiotics.

This local study reported high resistance to nitrofurantoin (100%). The data is consistent with published study of [14] who found that the resistance of *P. mirabilis* to this antibiotic was 76.5%.

Results also showed that five isolates (75%) were resistant to ampicillin and eight isolates were resistant to pipracillin (40%).These observations are in agreement with studies of [15]who found that (62%) of *Proteus* isolates were resistant to ampicillin and pipracillin respectively, and [16] reported that ampicillin has no more effect on any of the isolates of UTI. It was observed that the isolates of local study were resistant to penicillin.

In respect to the resistance to the third generation cephalosporins, the study showed that eight isolates (40%) were resistant to ceftazidime, and about the ceftotaxime was observed that resistance to this antibiotic scored (30%), while [8] reported (30%) resistance to cefotaxime and [17] observed (17.4%) resistance to ceftazidime of *Proteus* spp. which isolated from urine. The resistance to monobactam group, represented by aztreonam, it was revealed that four isolates (20%) showed resistance to this antibiotic. The resistance to the carbapenems was demonstrated by the resistance to imipenem with percentage (15%), Imipenem is uncommon to be used in our country therefore the antibiotic resistance is low.

In respect to aminoglycosides, this study demonstrated 50% resistance of *P. mirabiis*

isolates to gentamicin, while [18] reported that the resistance to gentamicin was (30%) in urinary isolates of *Proteus*.

Aminoglycoside is important in treatment of UTI and increasing resistance of gentamicin was due to frequent use of this drug in local hospitals. Low resistance to amikacin has been observed as 5% and this is result compatible with 1.6% of [18] Regarding quinolones resistance, results indicated that (40%) of the isolates were resistant to ciprofloxacin, while [19] reported that the resistant to ciprofloxacin was (93%), but [15] detected that the antibiotic resistance was (12.Also the study indicated that (27%) of the isolates were resistant to nalidixic acid. This agrees with [14] who observed the resistance of the isolates to nalidixic acid was 18%. The isolates have 65% resistance to Trimethoprim/ sulfamethoxazole.

Multi-drug resistance to *Proteus* isolates could be a result of the extra outer cytoplasmic membrane which contains a lipid bilayer, lipoproteins and lipopolysaccharide [20]. Resistance of *Proteus* to antibiotics was due to selection for drug resistance has been associated with an increased and inappropriate use of antibiotics .There is an irregular use of antimicrobial agents in Iraq.

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