

Research Article

Antimicrobial Resistance Patterns and Extended Spectrum *Beta-lactamases* Producing by *Proteus mirabilis* Isolated from Different Sources

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Abstract

A total of 801 samples included 179 clinical samples and 622 animal samples were collected from Baghdad province. All samples were cultured on blood agar and MacConkey agar plates to isolate *Proteus mirabilis* bacteria. Results showed that rate isolates of *P. mirabilis* from clinical and animal samples were 25.89% (51/179) and 5.95% (37/622) respectively. Antibiotic susceptibility test showed that lowest resistance rates for clinical *P. mirabilis* isolates were 3.9% for ciprofloxacin, 7.8% for norfloxacin, 9.8% for imipenem, 13.7% for levofloxacin and 15.7% for cefotaxime, and highest resistance rates were 82.4% for cefepime, 78.4% for piperacillin, 56.9% for ceftazidime and 54.9% for cefoxitin. In regards to animal isolates, they were 100% sensitive to cefoxitin and 100% resistance to piperacillin. Their resistance rates were 2.7% to amikacin, 5.4% to ciprofloxacin and 8.1% to cefepime, imipenem and levofloxacin. The results revealed that all *P. mirabilis* isolates were 100% multidrug resistance for 2-8 antibiotics. Extended spectrum β -lactamases produced were detected in 52.94% of clinical *P. mirabilis* isolates and in 48.65% of animal isolates.

Keywords: *Proteus mirabilis*, antibiotic, extended-spectrum β -lactamase.

الخلاصة

جمعت 801 عينة تضمنت 179 عينة سريرية و 622 عينة حيوانية من محافظة بغداد تم زرعها على وسطى آكار الدم وآكار الماكونكي لعزل البكتريا *Proteus mirabilis*. أظهرت النتائج أن معدل عزل *P. mirabilis* من العينات السريرية كان 25.89% (51/179) وأن معدل عزلها من العينات الحيوانية كان 5.95% (37/622). أظهرت نتائج فحص الحساسية أن أقل معدل مقاومة لعزلات *P. mirabilis* السريرية كان لمضادات ciprofloxacin (3.9%), norfloxacin (7.8%), levofloxacin (13.7%), imipenem (9.8%) و cefotaxime (15.7%) وأن أعلى مقاومة كانت لمضادات cefepime (82.4%), piperacillin (78.4%), ceftazidime (56.9%) و cefoxitin (54.9%). وفيما يخص العزلات الحيوانية وجد أن جميعها كانت حساسة (100% حساسة) لمضاد cefoxitin بينما كانت جميعها مقاومة (100%) لمضاد piperacillin، وكان معدل المقاومة لـ ciprofloxacin (5.4%), amikacin (2.7%) ولكل من cefepime و imipenem و levofloxacin (8.1%). وبينت النتائج أن جميع العزلات البكتيرية امتلكت المقاومة المتعددة لـ 2-8 من المضادات. وقد وجد أن 52.94% من عزلات *P. mirabilis* السريرية و 48.65% من العزلات الحيوانية كانت منتجة لأنزيمات البيتا لاكتيميز واسعة الطيف.

Introduction

Proteus mirabilis is a rod shaped, Gram-negative, facultative anaerobic, nonlactose fermenter, belongs to the family enterobacteriaceae, inhabits human colon as part of human intestinal flora and it is also found in soil and water [1]. *Proteus* species are distinguishable from most other genera by their swarming motility on surface of solid culture

media [2] and it causes urinary tract infections in patients with long-term indwelling catheters or individuals with abnormalities (functional or structural) of the urinary tract [3].

P. mirabilis is an opportunistic organism causes nosocomial urinary tract infections (46%), surgical wounds (24%) and lower respiratory tract (30%) and implicated in meningitis, empyema, osteomyelitis and gastroenteritis [4]. Patients with urinary tract infections caused by *P.*



mirabilis often develop bacteriuria, kidney and bladder stones, catheter obstruction due to stone encrustation and acute pyelonephritis [5].

In our country, we see an increase in the number of multidrug resistant Gram-negative bacteria. Due to increasing antibiotic resistance in the family enterobacteriaceae the multidrug resistant *P. mirabilis* isolates were recorded as worldwide [6]. Information from the world indicates a decrease in the total stock of antibiotics effectiveness, resistance to all first-line and last-resort antibiotics is increased [7].

Extended spectrum beta lactamases (ESBLs) have emerged as a major threat worldwide with limited treatment option [8]. ESBLs are enzymes that mediate bacterial resistance to most beta-lactam antibiotics, including penicillins, first-, second-, and third-generation cephalosporins, and aztreonam (not the cephamycins or carbapenems). They are inhibited by β -lactamase inhibitors such as clavulanic acid, tazobactam or sulbactam. *Beta-lactamases* are classified by Bush *et al.* (1995) into 4 major groups based on functional properties of enzymes (i.e. the substrate and inhibitor profiles). ESBLs belong to group 2be in this classification scheme [9].

Evolution and spread of a multidrug-resistant *Proteus mirabilis* with chromosomal AmpC-type beta-lactamase were reported in Europe [10].

Several local researches revealed that clinical *P. mirabilis* isolates from different anatomical sites are ESBLs producer [11-12]

The present study was aimed to isolate *P. mirabilis* from various clinical and animal sources and comparing between their antimicrobial patterns and extended spectrum β -lactamases production.

Materials and Methods

Collection of Samples

A Total of 801 samples were collected from patients and animals in Baghdad province during a period from 26/10/2015 to 29/3/2016.

179 clinical samples were collected from patients attended to Al-Yarmouk Teaching Hospital, Specialist Burns Hospital and Protect Children Specialist Hospital. The clinical samples were distributed as follow: (61) urine, (68) ears swabs and (50) wounds swabs.

While 622 animal samples were collected from different animals, including (462) chicken faeces were collected from two poultry farms located in

Al-Sha'ab and Al-Taji, (100) cows rectal swabs, (50) cats rectal swabs and (10) dogs rectal swabs were collected from Baghdad Veterinary Hospital, Agriculture Directorate\Veterinary Research and Laboratories Department, and Veterinary Laboratories at Al-Sinak.

Samples Procedures

Urine Samples

61 mid-stream urine samples were collected from patients under a septic condition in a sterile container. The samples were streaked with out flaming loop on blood agar and MacConkey agar plates [13].

Ear Swabs

68 ear swabs were collected from patients with otitis media. Swabs were streaked on blood agar and MacConkey agar plates [14].

Wound swabs

50 wound swabs were collected from different wound infections. Swabs were streaked on blood agar and MacConkey agar plates [15]

Chicken Faeces Samples

462 fresh samples of chicken faeces were collected in conditions prevent contaminations. The samples were inoculated on blood agar and MacConkey agar plates [16].

Animal Rectal Swabs

100 cows rectal swabs, 50 cats rectal swabs and 10 dogs rectal swabs were obtained by using transport media swabs. Swabs were streaked on blood agar and MacConkey agar plates. Suspected *Proteus* isolates were isolated and identified.

Identification of Bacterial Isolates

Cultural Characteristics

Swarming motility of bacterial isolates on blood agar medium and non-lactose fermenting colonies on MacConkey agar were observed.

Microscopic Examination

Gram stained smears of bacterial isolates were examined under light microscope to observe cell morphology, cells arrangement, reaction with Gram-stain and spore forming.

Biochemical Tests

Several biochemical testes were done to identify the bacteria *p. mirabilis* isolates included:

catalase, oxidase, IMViC tests, urease, glucose fermentation and H₂S production [17].

Vitek-2 Compact System

The identification of *P. mirabilis* isolates was confirmed by using Vitek-2 compact system according to the manufacturer's instruction.

Antibiotic Susceptibility Test (AST)

The susceptibility of *P. mirabilis* isolates to antibiotics was done by Kirby-Bauer disk diffusion method [18]. The following antibiotics used were: amoxicillin/clavulanic (20/10µg), piperacillin (100µg), cefoxitin (30µg), cefotaxime (30µg), ceftazidime (30µg), cefepime (30µg), aztreonam (30µg), imipenem (10µg), meropenem (10µg), amikacin (30µg), gentamicin (10µg), doxycycline (30µg), norfloxacin (10µg), ciprofloxacin (5 µg) and levofloxacin (5µg). The turbidity of bacterial isolates suspensions was adjusted to 0.5 McFarland turbidity and spread evenly on the surface of Muller-Hinton agar plates with sterile cotton swabs. The antibiotic disks were applied to the surface of inoculating agar plates. Then plates were incubated aerobically at 37° C for 24 hours. The inhibition growth zones diameters around the antibiotic disks were measured by a rule. The results were compared to standard criteria in Clinical Laboratory Standards Institute, CLSI [19]

Extended spectrum *Beta-lactamases* (ESBLs) production

All *P. mirabilis* isolates were tested to ESBLs production by the double-disk synergy test (DDST) as follow: The inoculum was adjusted to 0.5 McFarland standard. The swab was dipped into the bacterial suspension and pressed against internal side of tube to remove excess fluid. The wet swab was evenly streaked on Muller-Hinton agar plate, thereafter an amoxicillin/clavulanic acid disk (20/10µg) was placed at the center of the plate, then ceftazidime, cefotaxime, aztreonam, and piperacillin were placed around the central amoxicillin/clavulanic acid disk at distances of 22-25 mm (center to center) from a disk containing amoxicillin/clavulanate. The plates were incubated at 37° C aerobically for 18-24 h. The test is considered positive when the zone of inhibition of any of the antibiotics is

larger towards the amoxicillin/clavulanate disk [20].

Statistical Analysis

Chi-square test was used to test the results obtained for the significance.

Results and Discussion

Isolation and Identification

Out of 801 clinical and animal samples 88 (51 clinical+37 animal) isolates were identified as *P. mirabilis* depending on morphological and cultural properties as well as conventional biochemical tests.

All bacterial isolates were swarmed on blood agar medium, non-lactose fermenting colonies, polymorphic Gram-negative rods and non-spore formers, catalase positive, oxidase and indole negative, methyl red positive, Voges-Proskauer negative, citrate utilization variable, urease positive, glucose fermenting and H₂S production [17]. Then identification of *P. mirabilis* was confirmed by using Vitek-2 compact system.

Prevalence of *Proteus mirabilis*

The results in Table 1 showed that rate isolate of clinical *P. mirabilis* was 28.49% (51) from various clinical samples showed that following distribution: 21 (34.43%) urinary isolates, 16 (23.53%) ears isolates and 14 (28%) wounds isolates.

Al-Bassam and Al-Kazaz (2013) mentioned that rate isolate of urinary *P. mirabilis* was 38% and rate isolate of wounds *P. mirabilis* was 24% [21], while Hassen (2008) reported that rate isolate of urinary *P. mirabilis* was 46.32% [22]. In the same trend Ali and Yousif (2015) revealed that rate isolate of urinary *P. mirabilis* was 17.64% [23]. O'Hara *et al.* (2000) mentioned that *P. mirabilis* accounts approximately 3% of nosocomial infection [24]. Al-Azawy *et al.* (2015) reported that rate isolate of ears *P. mirabilis* was 7% and rate isolate of urinary *P. mirabilis* was 24.24% [24]. Muluye *et al.* (2013) reported that predominant proteus species in ear infections was 27.5% [26]. Mordi and Momoh (2009) mentioned that wounds *P. mirabilis* accounts 17.5% [27]. In the same trend

Mohamed *et al.* (2013) revealed that rate isolate of wounds *P. mirabilis* was 14.5% [28].

Table 1: Source and number (%) of clinical *P. mirabilis* isolates

Isolation source	No. of samples	nb. of <i>P. mirabilis</i> isolates	% of total samples	% of each isolation source
Urine	61	21	11.73	34.43
Ear swabs	68	16	8.94	23.53
Wound swabs	50	14	7.82	28
Total	179	51	28.49	

The results in Table 2 showed that animal *P. mirabilis* isolates accounts (37) 5.95% from various animal samples showed that following distribution: 20 (4.33%) isolates from chicken faeces, 10 (10%) isolates from cows rectal

swabs, 5 (10%) isolates from cats rectal swabs and 2 (20%) isolates from dogs rectal swabs.

This finding was in contrast with Mansouri and Pahlavanzadeh (2009) whom found that rate isolate of *P. mirabilis* from chickens skin was 27.42% [29] and Nahara *et al.* (2014) who mentioned that rate isolate of *P. mirabilis* from chickens dropping was (27) 38.6% [16]. These differences in the percentage of isolation may be due to geographic region, isolate sources and size of samples.

Antibiotic Susceptibility Test

Table 3 and Figure 1 show the antibiotic resistant pattern of clinical and animal *P. mirabilis* isolates (51 clinical and 37 animal isolates) to fifteen selected different antibiotics. Results showed that there are differences to antibiotics resistance among clinical and animal *P. mirabilis* isolates, and all of the bacterial isolates were multidrug resistance.

Table 2: Source and number (%) of animal *P. mirabilis* isolates

Isolation source	No. of samples	No. of <i>Proteus mirabilis</i> isolates	% of total samples	% of each isolation source
Chicken faeces	462	20	3.22	4.33
Cow rectal swabs	100	10	1.61	10
Cat rectal swabs	50	5	0.80	10
Dog rectal swabs	10	2	0.32	20
Total	622	37	5.95	

Table 3: The percentage of antimicrobial patterns of clinical and animal *Proteus mirabilis* isolates.

Antibiotic µg/disk	Clinical <i>P. mirabilis</i> (n=51)		Animal <i>P. mirabilis</i> (n=37)	
	Resistant (%)	Sensitive (%)	Resistant (%)	Sensitive (%)
Amoxicillin/Clavulanate (20+10 µg)	24 (47.6)	27 (52.94)	21 (56.8)	16 (43.2)
Piperacillin (100µg)	40 (78.4)	11 (21.6)	37 (100)	0.0 (0.0)
Cefoxitin (30 µg)	28 (54.9)	23 (45.1)	0.0 (0.0)	37 (100)
Cefotaxime (30µg)	8 (15.7)	43 (84.3)	16 (43.2)	21 (56.8)
Ceftazidime (30µg)	29 (56.9)	22 (43.1)	16 (43.2)	21 (56.8)
Cefepime (30µg)	42 (82.4)	9 (17.6)	3 (8.1)	34 (91.9)
Aztreonam (30µg)	15 (29.4)	36 (70.6)	12 (32.4)	25 (67.6)
Meropenem (10µg)	24 (47.1)	27 (52.9)	18 (48.6)	19 (51.4)
Imipenem (10 µg)	5 (9.8)	46 (90.2)	3 (8.1)	34 (91.1)
Doxycycline (30µg)	22 (43.1)	29 (56.9)	11 (29.7)	26 (70.3)
Amikacin (30µg)	19 (37.3)	32 (62.7)	1 (2.7)	36 (97.3)
Gentamicin (10µg)	21 (41.2)	30 (58.8)	7 (18.9)	30 (81.1)
Ciprofloxacin (5µg)	2 (3.9)	49 (96.1)	2 (5.4)	35 (94.6)
Norfloxacin (10µg)	4 (7.8)	47 (92.2)	4 (10.8)	33 (89.2)
Levofloxacin (5µg)	7 (13.7)	44 (86.3)	3 (8.1)	34 (91.9)

Results in Table 3 and Figure 1 showed that the resistance rate of the most active antibiotics toward clinical *P. mirabilis* isolates were 3.9% (2) ciprofloxacin, 7.8% (4) norfloxacin, 9.8% (5) imipenem, 13.7% (7) levofloxacin and 15.7% (8) cefotaxime. They showed highly resistant rate to cefepime (82.4%), piperacillin (78.4%), ceftazidime (56.9%) and ceftazidime (54.9%). Whereas resistance rate to Amoxicillin/Clavulanate, Aztreonam, Meropenem, doxycycline, Amikacin and gentamicin was ranged from 16-50%. Chi-square statistical analysis showed that there is no significant difference between clinical and animal *P. mirabilis* antibiotic resistance ($p=0.361$).

Hassen 2008 mentioned that sensitive rate of clinical *P. mirabilis* to cefotaxime was 66.6%, gentamycin (73.65), ciprofloxacin (79%) and norfloxacin (75%) [22]. Ahmed 2015 reported that rate sensitive of *P. mirabilis* to cefotaxime and gentamycin was 65%, piperacillin 60%, ceftazidime and amikacin 30%, cefepime 20% [30]. Al-Azaway *et al.* (20015) reported that resistance rate of clinical *P. mirabilis* to ciprofloxacin was 10.8%, imipenem (16.2%) and amikacin (43.2%) [25].

In regards to animal *P. mirabilis* isolates, all isolates (100%) were sensitive to ceftazidime and all isolates were 100% resistance to piperacillin as shown in Table 3 and Figure 1.

The animal isolates showed low antibiotic resistance towards amikacin (2.7%), imipenem and cefepime (3.8%), ciprofloxacin (5.4%), levofloxacin (8.1%) and norfloxacin (10.8%). But they showed high resistance to amoxicillin/clavulanate (56.8%), meropenem (48.6%), cefotaxime and ceftazidime (43.2%). The percentage of resistance was 32.4% for aztreonam, 29.7% for doxycycline, and 18.9% gentamicin. Figure 1 shows the resistance rate of *P. mirabilis* animal isolates, (e.g., piperacillin, amoxicillin/clavulanate, cefotaxime, aztreonam, meropenem, ciprofloxacin and norfloxacin), was higher than clinical isolates.

Extended Spectrum Beta-lactamases

All *P. mirabilis* isolates (88 isolates) were screened for extended-spectrum β -lactamases production by the double-disk synergy test (DDST). It was noticed that high frequency of *P. mirabilis* producing ESBLs among both clinical and animal isolates.

The results in Table 4 indicated that 52.94% (27/51) of clinical *P. mirabilis* was ESBLs producing as follow: 12 (44.44%) urinary isolates, 8 (29.63%) ears isolates and 7 (25.93%) wounds isolates. Chi-square statistical analysis showed that there is no significant difference between clinical and animal *P. mirabilis* ESBLs producing ($p=0.691$).

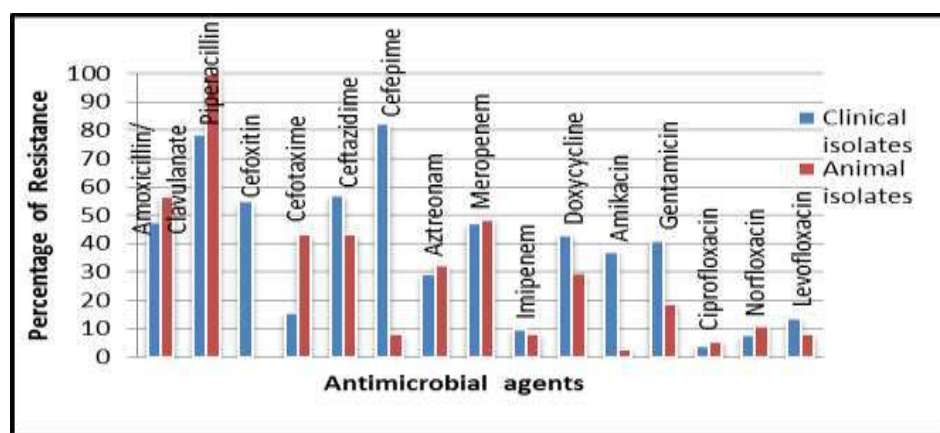


Figure 1: Percentage of antimicrobial resistance patterns of clinical and animal *P. mirabilis* isolates.

Hussein 2011, mentioned that 34.6% (18/52) of urinary *P. mirabilis* was ESBLs producing [12]. Al-Duliami *et al.* reported that 33.4% (8/24) of ears *P. mirabilis* was ESBLs producing [11]. Al-

Azaway *et al.* (2015) reported that 85.71% and 50% of *P. mirabilis* isolated from ears and wounds infection were ESBLs producing respectively [25]. Kanayama *et al.* 2010,

revealed that 37.8% (28/74) of clinical *P. mirabilis* was ESBLs producing [31].

Table 4: Number and percentage of clinical *P. mirabilis* isolates producing extended spectrum *Beta-lactamases* (ESBLs)

Source of isolates	No. of isolates	ESBLs producer	
		No. of isolates	Percentage ¹
Urine	21	12	44.44
Ear swabs	16	8	29.63
Wound swabs	14	7	25.93
Total	51	27	100

¹ Percentage calculated from total *P. mirabilis* isolates producing ESBLs.

Table 5: Number and percentage of *P. mirabilis* isolates producing extended spectrum *Beta-lactamases* (ESBLs)

Source of isolates	No. of isolates	ESBLs producer	
		No. of isolates	Percentage ¹
Chicken faeces	20	12	66.67
Cow rectal swabs	10	3	16.67
Cat rectal swabs	5	3	16.67
Dog rectal swabs	2	0	0
Total	37	18	100

¹ Percentage calculated from total *P. mirabilis* isolates ESBLs.

Results are also showed that 48.65% (18/37) of animal *P. mirabilis* was ESBLs producing as follow: 12 (66.67%) isolates from chicken faeces, 3 (16.67%) isolates from cows rectal swabs, 3 (16.67%) isolates from cats rectal swabs, and the two dogs rectal isolates were non ESBL producers (see Table 5).

Conclusion

All *P. mirabilis* isolates were multidrug resistance to 2-8 antibiotics; the high resistance rate of *P. mirabilis* to antibiotics is reflecting the resistance level among enterobacteriaceae. ESBLs production among *P. mirabilis* isolates was high.

The statistical analysis of results obtained did not show significant correlation between antibiotic resistance or extended spectrum β -lactamases production and the origin of isolates.

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