Prevalence and Potential Risk Factors of Hospital Acquired Extended-Spectrum Beta-Lactamase—Producing Proteus Species

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Abstract
Background: Multidrug resistance and production of extended spectrum $\beta$-lactamases (ESBLs) by a large group of bacterial agents in hospitals are to be a matter of scientific concern. Objective: This cross-sectional study was aimed to investigate the prevalence of ESBL producing Proteus species and risk factors associated with hospital acquired infection in addition to study the antibiotics susceptibility patterns of all bacterial isolates from inpatients of four Yemeni general hospitals. Methods: A total of 740 consecutive non-repeat culture isolates were obtained from admitted patients of Al-Kuwait University Hospital, Al-Thowra General Hospital, Al-Jumhori Teaching Hospital, and Military General Hospitals Sana’a city. We used Kirby-Bauer disk diffusion method to detect antimicrobial susceptibility and establish the presence of ESBLs-producing bacteria according to the Clinical and Laboratory Standards Institute guidelines. Results: Out of 740 isolate, 233 (31.5%) were Escherichia coli followed by Staphylococcus aureus 188 (25.4%), Pseudomonas aeruginosa 149 (20.1%), Klebsiella sp. 107 (14.5%), Enterococcus faecalis 25 (3.4%) and Proteus spp. 38 (5.1%). The highest frequencies of ESBLs producing among Proteus sp. were Proteus mirabilis 26 out 38 (68.4%) and Proteus vulgaris 12 out 38 (31.6%). The most effective of antimicrobial susceptibility pattern among Proteus spp. were Imipenem (100%) followed by Pipracillin-Tazobactam (92.3%) for P. mirabilis and (83.3%) for P. vulgaris, while the Amikacin (80.8%) for P. mirabilis and P. vulgaris with (91.7%). Amoxicillin and Cefotaxime were the highest for both species (100%). Conclusion: The prevalence of ESBL-producing Proteus spp. detected in this study is of great concern for public health authorities and a strict adherence of infection control policies and procedures with continuous antibiotics resistance surveillance including antimicrobial management and routine detection of ESBL-producing isolates are very important to prevent nosocomial infections.
Keywords
Extended Spectrum β-Lactamases, Hospital Acquired Infection, Proteus Species, Yemen

1. Introduction

Worldwide, multidrug-resistant microorganism posing the greatest threat to human health is major public health threat at health care settings [1]. β-Lactam antibiotics are the commonly prescribed antibiotics to treat bacterial infections, especially at Yemeni general and private health setting. However, most Gram-negative bacteria produce β-lactamases enzymes which are their major defense mechanism against β-lactam antibiotics [2]. Extended-spectrum β-lactamases (ESBLs) are the type of β-lactamase enzymes produced by certain Gram-negative bacteria in the family of Enterobacteriaceae [3]. ESBL enzymes give the bacteria ability to resist Penicillins and Cephalosporins of the first, second, and third generations as well as Aztreonam through hydrolysis of these antibiotics [4] [5] and are encoded by mobile genetic elements [6]. Alarmingly, these genes code resistance to not only Cephalosporins and penicillin but also other antibiotics such as Aminoglycosides, Fluoroquinolones, Tetracyclines, Chloramphenicol, and Sulfamethoxazole/Trimethoprim [7].

ESBL-producing members of Enterobacteriaceae have gained attention in hospital settings because of the limited therapeutic options to treat them resulting in poor clinical outcome, low practicing of infection control and prevention policies and guidelines and their increasing capacity to cause hospital acquired infections [7]. Hospitalized patients easily acquire these bacteria and eventually act as reservoir [8]. E. coli, K. pneumoniae, and Proteus spp. are the most common ESBL-producing bacterial species, although other bacterial species in the families of Enterobacteriaceae and Pseudomonadaceae are also known to produce such enzymes [9]. Patients infected with ESBL-producing bacteria are likely not treated with beta-lactam antibiotics owing to the risks of treatment failure leading to death and amplified infectiousness [2] [10]. Therefore, early detection of these bacteria is important to control and prevent nosocomial outbreaks in hospital settings.

The prevalence of ESBL-producing bacteria is unknown in Yemen. Therefore, this current study aimed to determine the prevalence of the potential risk factor, β-lactamase of Proteus spp. isolated from patients of Yemeni general hospitals using a phenotypic detection procedure based on disk diffusion method. This method is the cheapest strategy to meet the local demands in resource constrained settings such as Yemen general hospitals which consider the very low income in the region compared to more expensive and inapplicable genotypic detection techniques [11] [12] [13]. This will offer evidence on the reality of ESBL prevalence in the Yemeni general hospitals environment and will
represent valuable help to control this emerging problem.

2. Materials and Methods

2.1. Study Area and Setting

A laboratory based cross-sectional study design was used to conduct this study from March 01, 2015-February 28, 2016 at Al-Kuwait University Hospital, Al-Thowra General Hospital, Al-Jumhori Teaching Hospital, and Military General Hospitals, Sana'a city. The 1200-bed capacity of the hospitals gives service for more than 3000 patients per day at its outpatient department. The 4 hospitals have about 1000 technical staff and 3500 non-technical staffs. The principal activity of the hospitals is provision of health care but it is also engaged in medical education and research. The Hospital is serving a catchment population of more than 5 million; from several provinces (Amran, AL-Mahweet, Hajjah, Rayma, Dhamar, Al-Baidha, IBB and Al-Hodaidah).

2.2. Sample Collection and Antimicrobial Susceptibility Test

Clinical samples of pus and urine were collected aseptically and processed at the microbiology laboratories of Al-Gumhori hospital Laboratory and Al-Aulaqi Specialized Lab. Collection, isolation and identification of bacterial isolates was conducted following standard bacteriological technique [14]. For isolation and identification of the following media were used: Blood agar, MacConky agar, XLD, TSI, KIA, SIM, Urea and Citrate. The bacterial isolates were then tested for antimicrobial susceptibility test by the disc diffusion method according to NCCLS [15]. The following antibiotics discs (HiMedia, Mumbai, India) were used in the sensitivity test; Amikacin (30 μg), Amoxicillin (10 μg), Amoxicillin-clavulanic acid (20/10 μg), Ceftazidime (30 μg), Cefotaxime (30 μg), Ceftriaxone (30 μg), Ciprofloxacin (30 μg), Imipenem (10 μg), Nalidixic acid (30 μg), Ofloxacin (5 μg), Pipracillin/Tazobactum (110/10 μg), Tetracycline (30UI) And Trimethoprim-sulfamethoxazole (25 μg).

2.3. Testing for the ESBL Production

The ESBLs detection was carried out by modified double disc synergism test using

![Figure 1.](image-url) (CAZ) Ceftazidime, (CTX) Cefotaxime, (CRO) Ceftriaxone, (AMC) Amoxicillin-Clavulanic acid.
(HiMedia, Mumbai, India), Cefotaxime and Ceftriaxone. All the strains which will show a diameter of less than 22 mm of Ceftazidime, 25 mm Ceftriaxone and 27 mm Cefotaxime were selected for checking the ESBLs production. The ESBL production was tested by the Modified Double Disc Synergy Test using a disc of Amoxicillin-Clavulanate (20/10 μg) along with 3 Cephalosporins (Ceftazidime, Cefotaxime and Ceftriaxone). A lawn culture of the organisms was made on a Mueller-Hinton agar plate, as recommend by CLSI [16]. A disc which contained Amoxicillin-Clavulanate (20/10 μg) was placed in the centre of the plate. The discs of third generation Cephalosporin and fourth generation Cephalosporin was placed 15 mm and 20 mm apart respectively, centre to centre to that of the Amoxicillin-Clavulanatedisc [17]. Any distortion or increase in the zone towards the disc of Amoxicillin-Clavulanate was considered as positive for the ESBLs production Figure 1.

2.4. Data analysis Method

Data were analyzed by SPSS statistical program version 21.0 and non normal distributed data were expressed by median and range. Nominal data were expressed by number and percentage differences and associations between categorical variables were tested by Chi-square test and considered statistically significant at P-value < 0.05 in addition the odds ratio (ORs) and 95% confidence interval (CI) were also calculated.

3. Results

A total of 740 clinical samples were tested for culture and antimicrobial sensitivity. Of the total samples processed 704 were found to be culture positive and Proteus spp. accounted 38/704 (5.1%) as shown in Figure 2. The majority of the isolates were collected from surgical wound swab and urine and the leading isolate were E. coli 233 (31.5%) followed by 188 (25.4%) S. aureus, 149 (20.1%) P. aeruginosa, 107 (14.5%) Klebsiella spp, 38 (5.1%) Proteus spp. and 25 (3.4%) of Enterococcus faecalis.

Figure 2. Bacterial isolates from clinical specimens.
The distribution of *Proteus* spp. isolates from admitted patients with the total 38 isolates, 26 (68.4%) *P. mirabilis* and 12 (31.6%) *P. vulgaris* of pus and urine samples as shown in Figure 3(a) and the prevalence of positive *Proteus* spp. ESBL producer were ESBL positive (13.2%) and (86.8%) were ESBL negative Figure 3(b).

The association of ESBLs producing *Proteus species* according to gender statically non-significant and the majority rate of ESBL-producer was obtained from male 4 (80%), while 1 (20%) were obtained from female as shown in Table 1.

The association and distribution of ESBLs producing *Proteus species* accord to the types of specimens statically non-significant. The majority rate of ESBL-producer was obtained from pus and wound swabs 4 (80%), while 1 (20%) were obtained from urine specimens as shown in Table 2.

![Figure 3](image)

**Figure 3.** (a) & (b). Distribution and prevalence of *Proteus spp.* positive extended spectrum β-lactamase isolated.

### Table 1. The association between sex and contracting ESBL producing *Proteus spp.*

| Character | ESBL Positive | ESBL Negative | Total | X²* | P-value | Odds Ratio | 95% Confidence Interval |
|-----------|---------------|---------------|-------|-----|---------|------------|-------------------------|
|           | NO. | %    | NO. | %   | NO. | %     |            |                      |
| Sex       |     |      |     |      |     |       |            |                      |
| Male      | 4   | 80   | 25  | 75.8| 29  | 76.3 | 0.6       | 0.837                 | 1.28                   | 0.12                   | 13.17                  |
| Female    | 1   | 20   | 8   | 24.2| 9   | 23.7 |           |                       |                        |                        |                        |
| Total     | 5   | 13.2 | 33  | 86.8| 38  | 100.0|           |                       |                        |                        |                        |

*chi square test.

### Table 2. The association between type of specimen and contracting ESBL producing *Proteus spp.*

| Character | ESBL Positive | ESBL Negative | Total | X²* | P-value | Odds Ratio | 95% Confidence Interval |
|-----------|---------------|---------------|-------|-----|---------|------------|-------------------------|
|           | NO. | %    | NO. | %   | NO. | %     |            |                      |
| Type of specimen |     |      |     |      |     |       |            |                      |
| Urine     | 1   | 20   | 13  | 39.4| 14  | 36.8 | 0.702     | 0.633                 | 0.385                   | 0.039                   | 3.83                    |
| Pus       | 4   | 80   | 20  | 60.6| 24  | 63.2 |           |                       |                        |                        |                        |
| Total     | 5   | 13.2 | 33  | 86.8| 38  | 100.0|           |                       |                        |                        |                        |

*chi square test.
Table 3. The association between Length of hospital stay (days) and contracting ESBL producing Proteus spp.

| Character                  | ESBL          |          |          |          |          |          |          |          |          |
|---------------------------|---------------|----------|----------|----------|----------|----------|----------|----------|----------|
|                           | Positive      | Negative | Total    |          |          |          |          |          |          |
|                           | NO.           | %        | NO.      | %        | NO.      | %        | X²        | P-value  |
| 3 - 7                     | 0             | 0.0      | 10       | 30.3     | 10       | 26.3     | 3.42      | 0.136    |
| 8 - 14                    | 2             | 40.0     | 16       | 48.5     | 18       | 47.4     | 3.42      | 0.136    |
| >15                       | 3             | 60.0     | 7        | 21.2     | 10       | 26.3     | 3.42      | 0.136    |
| Total                     | 5             | 13.2     | 33       | 86.8     | 38       | 100.0    | 3.42      | 0.136    |

Table 4. The multiple drug resistance patterns the ESBL-producing *P. mirabilis* and *P. Vulgaris* to the commonly antibiotics (*S* = Sensitive *R* = Resistance).

| Antibiotics               | *P. mirabilis (n = 26)* |          |          | *P. vulgaris (n = 12)* |          |          |
|---------------------------|-------------------------|----------|----------|-----------------------|----------|----------|
|                           | **S**                   | **R**    | **S**    | **R**     | **S**    | **R** |
|                           | No.     | %        | No.      | %        | No.      | %        | No.      | %        | No.      | %        |
| Imipenem                  | 26      | 100.0    | 0        | 0.0      | 12       | 100.0    | 0        | 0.0      |                     |
| Pipracillin-Tazobactam    | 24      | 92.3     | 2        | 7.7      | 10       | 83.3     | 2        | 16.7     |                     |
| Amikacin                  | 21      | 80.8     | 5        | 19.2     | 11       | 91.7     | 1        | 8.3      |                     |
| Tetracycline              | 12      | 46.2     | 14       | 53.8     | 6        | 50.0     | 6        | 50.0     |                     |
| Ofloxacin                 | 8       | 30.8     | 18       | 69.2     | 4        | 33.3     | 8        | 66.7     |                     |
| Ciprofloxacin             | 8       | 30.8     | 18       | 69.2     | 2        | 16.7     | 10       | 83.3     |                     |
| Trimethoprim-Sulfamethoxazole | 5       | 19.2    | 21       | 80.8     | 4        | 33.3     | 8        | 66.7     |                     |
| Amoxicillin Clavulanic acid | 4     | 15.4   | 22       | 84.6     | 1        | 8.3      | 11       | 91.7     |                     |
| Ceftriaxone               | 3       | 11.5     | 23       | 88.5     | 0        | 0.0      | 12       | 100.0    |                     |
| Ceftazidime               | 1       | 3.8      | 25       | 96.2     | 2        | 16.7     | 10       | 83.3     |                     |
| Nalidixic acid            | 2       | 7.7      | 24       | 92.3     | 0        | 0.0      | 12       | 100.0    |                     |
| Cefotaxime                | 0       | 0.0      | 26       | 100.0    | 0        | 0.0      | 12       | 100.0    |                     |
| Amoxicillin               | 0       | 0.0      | 26       | 100.0    | 0        | 0.0      | 12       | 100.0    |                     |

The highest ESBL positive rate according to length of hospital stay (days), statically non-significant was found among patients who had long hospitalization stay more than 15 day was (60.0%), followed by patients with moderate hospitalization stay from 8 - 14 day was (40.0%), finally no ESBL record at 3 - 7 hospital stay with (0.0%) as shown in Table 3.

The susceptibility patterns of *Proteus spp.* as shown in Table 4, the (100.0%) Imipenem was the most effective antibiotic in vitro for, *P. mirabilis* and *P. Vulgaris* followed by (92.3%) Pipracillin-Tazobactam for *P. mirabilis* and (83.3%) for *Proteus vulgaris*, while the (91.7%) Amikacin was more than effective for *P. vulgaris* and (80.8%) *P. mirabilis*. The relative effective of (46.2%) of Tetracycline for *P.
Table 5. Prevalence of phenotypic ESBL-producing *P. mirabilis* and *P. Vulgaris* by combined disc diffusion test (*S* = Sensitive, *R* = Resistance).

| Antibiotics               | ESBL Positive *P. mirabilis* n = 5 |
|---------------------------|-------------------------------------|
|                           | **S** | **R** |
|                           | **No.** | **%** | **No.** | **%** |
| Imipenem                  | 5     | 100.0 | 0       | 0.0   |
| Pipracillin-Tazobactam    | 5     | 100.0 | 0       | 0.0   |
| Amikacin                  | 4     | 80.0  | 1       | 20.0  |
| Tetracycline              | 2     | 40.0  | 3       | 60.0  |
| Ofloxacin                 | 2     | 40.0  | 3       | 60.0  |
| Ciprofloxacin             | 2     | 40.0  | 3       | 60.0  |
| Trimethoprim-Sulfamethoxazole | 1     | 20.0  | 4       | 80.0  |
| Amoxicillin Clavulanic acid | 1     | 20.0  | 4       | 80.0  |
| Ceftriaxone               | 0     | 0.0   | 5       | 100.0 |
| Ceftazidime               | 0     | 0.0   | 5       | 100.0 |
| Nalidixic acid            | 0     | 0.0   | 5       | 100.0 |
| Cefotaxime                | 0     | 0.0   | 5       | 100.0 |
| Amoxicillin               | 0     | 0.0   | 5       | 100.0 |

*mirabilis* and (50%) *P. vulgaris*. On the other hand, the highest resistance rate of (100.0%) Amoxicillin, and Cefotaxime for Proteus spp. The high resistance rate (88% - 100%) to all 3rd generations of cephalosporins followed by (92.3%) Nalidixic acid (84.6%) Amoxicillin-Clavulanic acid and (80.8%) Trimethoprim-Sulfamethoxazole.

Table 5, The results indicate that the Imipenem and Pipracillin-Tazobactam were the most effective antibiotic in vitro with the percentage (100.0%), followed by Amikacin (80.0%), then Tetracycline, Ofloxacin and Ciprofloxacin with percentage (40.0%). On the other hand, the result indicates a high resistance rate to Amoxicillin, Cefotaxime, Nalidixic acid, Ceftazidime and Ceftriaxone with the percentage (100.0%).

4. Discussion

Extended spectrum β-lactamases (ESBLs) are known to cause problems in patients who are especially hospitalized with an increase of prevalence during the exposure to different antibiotics. Consequently, this may result in treatment failure and death due to a delay of an adequate antimicrobial therapy. Worldwide, the incidence of ESBL *Enterobacteriaceae* in hospitals is dramatically increasing and the therapeutic option is very limited [3]. No published data are available on the prevalence of ESBL producing *Proteus spp* and its antimicrobial susceptibility patterns in Yemen. So this study is considered the first study that investigated the prevalence and antibiotics susceptibility patterns of ESBL pro-
Producing *Proteus spp* among inpatients of four public hospitals in Sana’a city, Yemen. So in the resourced constrained setting like Yemeni general hospitals, the use of combined disk diffusion technique for rapid phenotypic detection and identification of ESBL-producing bacteria is cheapest strategy to meet local demands due to war and siege [18] compared to more expensive and unaffordable genotypic detection techniques. Disk diffusion technique requires only necessary antibiotic discs and appropriate conventional culture media that are used routinely with other routine bacteriology works [19] [20]. The combined disk diffusion technique had a turnaround time of only 48 hours from the time of specimen culture to the final ESBL results, which is an advantage compared to other techniques [21]. Therefore, the family of *Enterobacteriaceae* ESBL-producing enzymes has become a concern in the treatment of infections and infection control programs at health care settings. Most of this family strains are resistant to ampicillin because of the production of plasmid mediated β-lactamase enzymes and some other isolates are susceptible to later generation Cephalosporins [5] [16] however, spontaneous mutations occurrence may result in novel β-lactamases which can inactivate extended-spectrum Cephalosporins and Penicillins. These β-lactamases are known as extended-spectrum β-lactamases (ESBLs) [22]. Recently, these enzymes have been found in other genera and species, such as *P. mirabilis, Salmonella spp.,* and *Enterobacter spp.* [23].

ESBLs-producing bacteria are a major problem in the management of certain bacterial infections and are a growing concern in those hospitals where antibiotics use is frequent and the patients are in critical conditions [7]. ESBL-producing bacterial pathogens limit the therapeutic options for the treatment; therefore, strategies for laboratory detection of ESBL-producing bacteria as well as antimicrobial susceptibility testing are important. However, despite the challenges involved with the technical sensitivity in the routine detection of ESBLs-producing bacteria, use of combined disk diffusion test is of great help in the resource limited country such as Yemeni general hospitals. This phenotypic technique based on disk diffusion is the cheapest strategy in the rapid detection of ESBLs to meet local demands in a resourced constrained setting. Therefore, in the current study, we detected presence of ESBL-producing bacteria using indicator antibiotics including Ceftazidime, Cefotaxime and Ceftriaxone using special screening zone or MIC breakpoints which helped in recognition of ESBL production. The selection of indicator antibiotics was based on their likelihood of being readily hydrolyzed by one of the many types of ESBL enzymes produced by the mentioned bacteria.

This study, prevalence of ESBLs-producing *Proteus spp* was found to be high 5.1% compared to the prevalence recorded from Saudi Arabia (3.0%) [24] (2.9%) [25] and India (3.04%) [26] and (1.12%) [27] but this result was low compared to those reported from Ghana (8.4%) [28] with the 68.4%, 31.6% rate among the *Proteus spp* (*P. mirabilis* and *P. vulgaris*) respectively which similar to Indian studies (62.2%) *P. mirabilis* & (32.5%) *P. vulgaris* reported by Syntem et al., [29] and Ghana (61.5%) P. mirabilis & (30.5%) *P. vulgaris* [28].
The association between sex prevalence of *Proteus spp* showed that the rate among males and (23.6%) female agreed with Saudi Arabia reported by [24] (75.0%) males and (25.0%) females. The prevalence of ESBL producing *Proteus spp* was 13.1% lower than reported from India 24% [29], 31.1% [30] and Ghana 72.9% [28] and higher than France 6.9% reported by De Champs et al., [31]. All of these findings support our hypothesis about the global spread of *Proteus spp* producing-ESBL. The risk factors associated with acquisition of ESBL high rate (30.0%) was found among patients who had long hospitalization stay more than 15 day, followed by (11.1%) among moderate hospitalization stay 8 - 14 although statically non significant. Regarding to the antibiotic susceptibility patterns of isolates, the highest antimicrobial activities against positive *Proteus spp* were observed and agreed with [24] reported from Saudi Arabia, and the antibiotics susceptibility test has revealed the most efficient antibiotics against *Proteus spp* were (91%) Imipenem, followed by (61.0%) Amikacin and Indian reported by Senthamarai et al., [26] where the (99.1%) Imipenem followed by Pipracillin-Tazobactam.

5. Conclusion

In a resourced constrained setting like Yemeni general hospitals, the use of phenotypical technique to identify and detect ESBL-producing bacteria based on combined disk diffusion is the cheapest strategy to routinely use to meet local demands compared with more expensive and unaffordable genotypic detection techniques. The prevalence of ESBL-producing *Proteus spp.* detected in this study is of great concern for public health care settings across Yemen, which requires sound and committed sustainable infection control measures including antimicrobial management and routine detection of ESBL-producing isolates.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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