ESKAPE pathogens among pediatric patients in Iraq

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Abstract

Background: ESKAPE pathogens are responsible for the common of nosocomial infections and capable of 'escaping' the biocidal action of antimicrobial agents. Aims: The aim of this study is to screen the prevalence of ESKAPE pathogens group among pediatric patients in Iraq. Methods: A total of 191 different clinical samples were collected from pediatric patients aged from 1 day till the age of 14 years old. Samples obtained included; blood, urine, CSF, burns wounds, and other data that were taken from patients before sampling. Results: Among 191 clinical samples, 12 isolates (6.3%) of ESKAPE Pathogens group were detected, S. aureus was recovered in high rate (33.4%) followed by P. aeruginosa, K. pneumoniae, and A. baumannii. However, no isolate belonged to E. faecium was recovered in this study. A high rate (75%) of isolates G-ve bacteria of ESKAPE pathogens group (P. aeruginosa, K. pneumoniae, E. cloacae, A. baumannii) were found to be ESBL-producers. Results also found that all S. aureus isolates were AmpC β-lactamase producers and all Gram-negative isolates within ESKAPE pathogens group were AmpC β-lactamase-producers. Different antibiotic susceptibility patterns were recorded among ESKAPE pathogens group isolates. MDR
was found in a high rate (75%), followed by PDR(16.6%), while XDR was detected only in one isolate (8.3%). **Conclusion:** S. aureus was recovered with a high rate among all ESKAPE pathogens group. CoNS are the most prevalent pathogens causing late-onset sepsis in neonates. ESBLs were predominant among G-veiESKAPE pathogens group isolates. All isolates in ESKAPE pathogens group were AmpC β-Lactamase producers.

**Keywords:** ESKAPE, pediatric Patients, Antibiotic Susceptibility, ESBL, AmpC


**Introduction**

One of the most important health concerns is antimicrobial resistance. The problem of nosocomial infection, the presence of multidrug-resistant bacteria in community and hospitals has been increased. High frequencies of multidrug-resistant bacteria have been grouped under the acronym ESKAPE: *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter spp.* (1). The majority of nosocomial infection and capable of escaping the biocidal action of antimicrobial agent are responsible with the ESKAPE pathogens (2,3). *Enterococcus faecium* possess intrinsic or acquired resistance to several antimicrobials, such as glycopeptides, β-lactams, and fluoroquinolones, and can show high levels of resistance to aminoglycosides (gentamicin and streptomycin), leading to drastically reduced therapeutic options for patients infected with enterococci, such that these bacteria are regarded as important pathogens with clinical relevance (4).

Studies on MRSA surveillance in hospitals and communities represent the main challenges of the healthcare setting (5). Unfortunately, penicillin resistant bacteria can spread in healthcare setting and in the community. Methicillin narrow spectrum semi-synthetic penicillin was introduced to overcome infections due to beta-lactamase-producing *S. aureus*. Strains from the hospital are named as hospital acquired *S. aureus*(HASA)(6,7).

Carbapenemase (KPC)-producing *Klebsiella pneumonia* (KPC-KP) were revealed to majorly contribute to the epidemic distribution of carbapenem-resistant Enterobacteriaceae, their spreading being mostly continued by strains of clonal complex 258 (ST-258 producing KPC-2 or KPC-3and ST-512 producing KPC-3)(8). *Acinetobacter baumannii* able to survive for extended periods of time in health care settings and can acquire resistance rapidly against antimicrobials. Multidrug resistant (MDR) A. baumannii strains are of global concern because these isolates are resistant to at least three classes of antibiotics (carbapenems, cephalosporins, aminoglycosides, fluoroquinolones) and thus limit treatment options(9).

Overexpression of efflux systems with wide substrate profiles is an important mutational mechanism in *P. aeruginosa*. Its impact on the resistance to antipseudomonal antibiotics (β-lactams, fluoroquinolones, aminoglycosides and polymyxin B)(10). Carbapenem-resistant *P. aeruginosa* is associated with the production of MBL and has the capability to hydrolyze all β-lactam antibiotics except aztreonam. It is responsible for nosocomial outbreaks in tertiary care centers (11,12). *Enterobacter cloacae* can produce the broad spectrum-beta-lactamase and the AmpC enzyme which lead to serious drug resistance, thus carbapenems are choice for the treatment of serious infections caused by ESBL- and AmpC-positive Enterobacteriaceae(13).
The aim of this study was to screen the prevalence of ESKAPE pathogens group among pediatric patients in Babylon province, Iraq.

**Materials and Methods**

**Study Design**

This cross-sectional study was designed to assess the prevalence of ESKAPE pathogens group among pediatric patients in Babylon province, Iraq. At the beginning of this study, 191 different clinical samples were collected during the period of July to November 2018 from the main two hospitals in Al-Hilla city (Babylon teaching hospital for maternity and children, mainly in nursery neonatal care unit (NNCU), intensive care unit (ICU), and general wards, and Hilla teaching hospital (mainly in burn unit).

**Samples collection:**

Clinical samples were obtained from pediatric patients aged from 1 day till the age of 14 years old who admitted to hospital. Samples obtained included; blood, urine, cerebrospinal fluid (CSF), burns wounds and others data like name, age, sex, address, body weight, attending for vaccination program before or not, any clinical documented infection or not, and whether received antibiotics before or not, were taken from patients before sampling.

**Ethical consideration:**

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. Verbal consent was taken from each patient parents before sampling. Investigative standards were rigidly preserved, primarily concerning confidentiality. Moreover, this study was undisclosed, participation of patients was optional, and verbal consent was received before data uptake process was started. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee (at College of Medicine, University of Babylon).

**Isolation and Identification Bacterial isolates:**

The presumptive detection of isolated bacteria was carried out by Gram staining, cultural characteristics of bacteria in special culture media such as Mannitol salt agar, Blood agar, and EMB agar. A single colony was taken from each primary positive culture. Its identification depended on the morphological properties. The colonies were then investigated by gram stain to observe bacterial cells. Specific biochemical tests were done to reach the final identification (14). The vitek2 system (AST N222 and AST-p580 cards) and API system strips were used to confirm the identification of the isolates.

**Antimicrobial Susceptibility:**

The antimicrobial susceptibility patterns of isolates to different antimicrobial agents was determined and interpreted according to CLSI, 2019 (15). The Vitek2 system, AST N222 and AST-p580 cards. Disk diffusion test was used to determination ESBLs and AmpC in G-ve bacteria with ESKAPE pathogens groups.

**Detection of AmpCβ-Lactamase**

**Initial Screening AmpCβ-Lactamase (Cefoxitin Susceptibility):**

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All isolates were tested for cefoxitin susceptibility by using standard disk diffusion method of Gram negative ESKAPE and Vitek2-system of MRSA (15). The resistant isolates (≤18mm inhibition zone diameter) were considered initially AmpC β-lactamase producers.

Extended-Spectrum β-Lactamase Production

All bacterial isolates that were β-lactamase producing were tested for ESBL production by initial screen test. The isolate would be considered potential ESBL producer, if the inhibition zone of ceftazidime, Ceftriaxone (30µg) disks was ≤ 22 mm (15). β-lactamase-producing isolates were tested for confirmatory ESBL production as follows:

**Disk Combination Test (CLSI, 2019)(15).**

The phenotypic confirmation of potential ESBL-producing isolates was performed by using disk diffusion method. Cefotaxime alone and in combination with clavulanic acid, Ceftazidime alone and in combination with clavulanic acid were tested. Inhibition zone of ≥ 5 mm increase in diameter for antibiotic tested in combination with clavulanic acid versus its zone when tested alone confirms an ESBL producing isolate.

**Determination of Antibiotic resistance patterns of ESKAPE pathogens:**

Antibiotic resistance patterns (MDR, XDR, and PDR) of all bacterial isolates belonged to ESKAPE pathogens (in this study), were determined using definitions of Magiorakos et al (16) as follows: 1-Multidrug resistance (MDR): Non-susceptibility to at least one agent in three or more antimicrobial categories; 2-Extensively drug resistant (XDR): Non-susceptibility to at least one agent in all but two or fewer antimicrobial categories; and 3-Pandrug-resistant (PDR): Non-susceptibility to all agents in all antimicrobial categories.

**Results**

**Prevalence of ESKAPE group among pediatric patients**

A total of 191 clinical samples were collected from pediatric patients [104 males (54.5 %) and 87 females (45.5%)] clinical samples at ranging age (1 days – 14 years) suffering from different infections.

Regarding distribution of pediatric patients according to the type of attendance (medical units), results found that out of 191 patients, 111 (58.1%) attended general wards, 35 (18.3%) attended burn wards, 27 (14.1%) from nursery neonatal care unit (NNCU), and 18 (8.9%) from intensive care unit (ICU) (Figure 1).

Among 191 clinical samples, 45 (23.5%) gave positive growth on ordinary culture media while 146 (76.4%) gave no growth. The reason of negative culture may be attributed to fungal infection, viral infection, or fastidious bacteria that might be lost during transporting. Results also revealed that out of 45 culture positive isolates recovered from pediatric patients, identified using biochemical tests, Vitek2-system ID cards, and API system high rate of isolation was found in urine isolates 16/45 (35.5%), followed by, wounds (burns) isolates 15/45 (33.3%), blood 13 (28.8%), while only one isolate of bronchial fluid (2.2%).
Among 45 (23.5%) positive growth of clinical samples, 17 bacterial isolates belonged to Coagulase-negative Staphylococci (CoNS) accounted for almost (37.7%), followed by *Escherichia coli* (22.2%), and *Staphylococcus aureus* (8.8%). Other bacterial species belonged to G-ve bacteria were isolated in different rates (Unpublished data). However, no isolate belonged to *E. faecium* was recovered in this study.

Results also revealed that out of 45 culture positive isolates recovered from pediatric patients, 12 pathogens of ESKAPE pathogens group were isolated. Out of 12 isolates, high rate of isolation was found in burns unit 6/12 (50%), followed by 3/12 isolates (25%) from general wards, 2/12 isolates (16.6) from intensive care unit, while only 1 isolate (8.3) was from nursery neonatal care unit (Table 1).

### Table 1: Distribution of ESKAPE pathogens group isolated from pediatric patients according to attendance site

<table>
<thead>
<tr>
<th>Attendance Site</th>
<th>Samples No.</th>
<th>Isolates No.</th>
<th>ESKAPE No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>General wards</td>
<td>111 (58.1%)</td>
<td>21 (46.6%)</td>
<td>3 (25.0%)</td>
</tr>
<tr>
<td>ICU*</td>
<td>18 (9.4%)</td>
<td>2 (4.4%)</td>
<td>2 (16.6%)</td>
</tr>
<tr>
<td>NNCU**</td>
<td>27 (14.1%)</td>
<td>6 (13.3%)</td>
<td>1 (8.3%)</td>
</tr>
<tr>
<td>Burns</td>
<td>35 (18.3%)</td>
<td>16 (35.5%)</td>
<td>6 (50.0%)</td>
</tr>
<tr>
<td>Sum</td>
<td>191 (100%)</td>
<td>45 (100%)</td>
<td>12 (100%)</td>
</tr>
</tbody>
</table>

*ICU (intensive care unit), **NNCU (nursery neonatal care unit).

Regarding distribution of ESKAPE pathogens group according to type of bacteria, *S. aureus* was high rate (33.4%), each of *P. aeruginosa* and *K. pneumoniae* have same rate (25%) and low rate (8.3%) belonged of each *E. cloacae* and *A. baumannii*, while *E. faecium* not isolated in this study.

The ESKAPE Pathogens group were mainly recovered from burn wounds, followed by blood Samples. However, No ESKAPE isolates were recovered from samples of Urine and CSF. In present study, the ESKAPE pathogens group corresponded 12 samples positive growth results high rate in burns wound 6 (50%), followed by blood 5 (41.6%) and bronchial fluid 1 (8.3%).
All ESKAPE isolates resistant to FOX Cefoxitin and 75% of isolates resistant to CAZ Ceftazidime, AMC Amoxicillin clavulanic acid, CRO ceftriaxone, ATM Aztreonam, AmpC β-lactamase, ESBL extended spectrum β-lactamase, ND (not determined).

Results of the present study found that 6/8 (75%) of the isolates G-ve of ESKAPE pathogens group were ESBL producers during the initial screening using ceftazidime disk which considered as suspected of ESBL-producing Gram-negative of ESKAPE pathogens group (Table 2). The initial detection of ESBL producing isolates was confirmed using disk combination method.

### Table (2): Antibiotic susceptibility by disk diffusion test for detection AmpC and ESBLs of Gram negative of ESKAPE pathogens group

<table>
<thead>
<tr>
<th>Bacterial Isolate</th>
<th>AmpCβ-lactamase</th>
<th>ESBLs</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S.aureus</em> W0075</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td><em>S.aureus</em> W0079</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td><em>S.aureus</em> W0018</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td><em>S.aureus</em> B00115</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td><em>K.pneumoniae</em> B0071</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>K.pneumoniae</em> W0077</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>K.pneumoniae</em> B0096</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>A. baumannii</em> B00119</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>P.aeruginosa</em> W0074</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>P.aeruginosa</em> W0076</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>P.aeruginosa</em> bro00138</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>E. cloacae</em> B0022</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

In present study, the production of AmpCβ-lactamases in β-lactam resistant of *S.aureus* isolates was detected by Vitek 2 compact while it was detected by DDT test in β-lactam resistant of Gram-negative isolates. The results in this regard revealed that all 12 ESKAPE pathogens group isolates were resistant to cefoxitin as primary screening marker for AmpC β-lactamase production.

Regarding distribution of Gram positive and Gram negative of ESKAPE pathogens group according to type of antibiotic resistance patterns, high rate of MDR 9/12 (75%) was detected, followed by PDR 2/12 (16.6%), while XDR was detected only in one isolate 1/12 (8.3%) (Figure 2).
Discussion

Regarding distribution of pediatric patients according to the type of attendance (medical units), results found that out of 191 patients, 111 (58.1%) attended general wards, 35 (18.3%) attended burn wards, 27 (14.1%) from nursery neonatal care unit (NNCU), and 18 (8.9%) from intensive care unit (ICU) (Figure 1).

The presence of low rate of pediatric patients attendance at intensive care unit (ICU) in this study may be due to the low numbers of beds available at the site of the study (hospital for maternity and children) which may leads consequely to low numbers of patients attendance.

However, several studies found that ICU and burn unit are the most medical units that have high rate of risk factor for infection (17-23).

Nosocomial infections (NIs) varies in different regions, for example Abdel-Wahab et al(18) in Egypt, reported that the rate of NIs were almost three times higher than any other departments of hospital and the incidence of NIs in the NICU was 21.4%. Pourakbari et al. (20) in Iran reported the rate of these infections to be 36% in PICU.

Results regarding of the isolated organisms from pediatric patients admitted to the burn unit In this study 16 (35.5%) while Coetzee et al(21) found that the rate 44.81% of the isolated organisms from pediatric patients admitted to the burn unit.

Results regarding attendance of pediatric patients in burn unit in this study 35 (18.3%) while Behzadnia et al(22) in Iran found that out of the total number of 34556 hospitalized patients in three teaching hospitals, 61 (0.17%) patients were children under 12 years old age with nosocomial infection from which 50.81% were females and 49.18% were males. Most of these patients 55.73% were admitted to the burn unit, while they found that 26.20% of NIs patients were admitted to NICU/PICU and respiratory infection 43.75% of NIs cases was the most common NIs in this ward.
followed by UTI, Blood infection and wound infection. Due to frequent airway suctioning, contamination of nurses’ hands is major causes of respiratory NIs in this ward.

Results of the present study revealed that ESKAPE Pathogens were mainly recovered from burn wounds (50%), followed by blood Samples (41.6%). However, No ESKAPE isolates were recovered from samples of Urine and CSF. Burn infection is an important cause of morbidity and mortality in hospitalized burn patients. The rate of nosocomial infections is higher in burn patients due to different factors like nature of burn injury itself, immunocompromised case of the patient(24). In addition, cross-infection results between various burn patients due to overcrowding in burn wards(25). Burn wound infections are largely hospital acquired and the infecting pathogens vary from one hospital to another(26). The burn wound represents a susceptible site for opportunistic colonization by organisms of endogenous and exogenous origin; thermal injury destroys the skin barrier that normally inhibits invasion by microorganisms. This makes the burn wound the most recurrently origin of sepsis in these patients(27).

The rate of blood infection was less common from burn wound(41.6%). The rate of blood infection in developing countries is 3 to 20 times higher than the other countries, and more than half of the hospitalized infants in NICUs are affected by blood infection(28). A full period of infancy consist of the first four weeks of term infants or four weeks after a preterm infant leaves the hospital(29). Neonatal sepsis is the disease of the infants less than a month (28 days) having clinical symptoms, and their blood culture is positive.

Fluid infection was less common compared with burn wounds and blood samples, it was rate 1/12 (8.3%). However, No ESKAPE isolates were recovered from samples of Urine and CSF.

Until 2002 such a genetic transfer was not reported for wild S. aureus strains. In 2002, a VRSA strain was isolated from the catheter tip of a diabetic, renal dialysis patient in Michigan (30). The isolate contained the mecA gene for methicillin resistance. Vancomycin MICs of the VRSA isolate were consistent with the VanA phenotype of Enterococcus species, and the presence of the vanA gene was contended by polymerase chain reaction. From 2002 to 2010, ten additional VRSA isolates were reported, eight from the United States, one from Iran, and one from India (31).

Karlowsky et al. (32) found that Gram-negative ESKAPE pathogens (K.pneumoniae, A.baumannii, P. aeruginosa, and Enterobacter species) are important etiologic agents of nosocomial infection that are frequently resistant to broad-spectrum antimicrobial agents. Gram-negative ESKAPE pathogens were collected from hospitalized patients in 11 Latin American countries from 2013 to 2015 as part of the Study for Monitoring Antimicrobial Resistance Trends (SMART) global surveillance program.

Acinetobacter baumannii is an opportunistic bacterial pathogen with the strong of causing hospital acquired infections, individually in intensive care units, urinary tract infection, meningitis, bacteremia, pneumonia, wound infections, healthcare-related infections (HAI’s) with multiple outbreaks (33,34).

Pseudomonas aeruginosa is a bacterial pathogen that is responsible for a wide range of infections in humans that can be associated with significant morbidity and mortality (35). This opportunistic pathogen mainly affects immunocompromised patients: it can be isolated from 50% to 60% of hospitalized patients (especially initiate in burns and scabs), as opposed to 1.2–6% of healthy individuals (36).

The ESKAPE pathogens group resistant to β-lactam antibiotic are suspected to be highly producers of ESBL’s; therefore, all were subjected to ESBLs production test. Performance of the test isolates in the ESBL initial screen disk test was assessed using ceftazidime disks.

Results of the present study found that 6/8 (75%) of the isolates G-ve of ESKAPE pathogens group were ESBL producers during the initial screening using ceftazidime disk (Table 2), which considered as suspected of ESBL-
producing Gram-negative of ESKAPE pathogens group. The initial detection of ESBL producing isolates was confirmed using disk combination method.

Extended spectrum β-lactamase (ESBLs) are enzymes found in gram-negative bacilli that mediate resistance to extended-spectrum cephalosporins and aztreonam (37). Al-Charrakhet al. (38) found that out of 88 K. pneumoniae strains were isolated from different samples in Iraq. B-lactamase-producing Klebsiella strains showed multiple-drug resistance. Klebsiella strains were also tested for their ability to produce ESBLs, 8 (21%) were ESBL-producers.

In present study, the production of AmpC β-lactamasin β-lactam resistant of S.aureus isolates was detected by Vetik2 compact while it was detected by DDT test in β-lactam resistant of Gram-negative isolates. The results in this regard revealed that all 12 ESKAPE pathogens group isolates were resistant to cefoxitin as primary screening marker for AmpC β-lactamase production (Table 2).

Although, some of AmpC types producing Gram-negative bacteria are susceptible to cefoxitin (39). In general, cefoxitin readily detects hyper-production of AmpC in some Enterobacteriaceae. A low level of production yields negative results or marginally positive results. In a previous study, in India, Manchanda and Singh (40), mentioned that 61% of AmpC β-lactamase producers were found to be resistant to cefoxitin and 39% of them were susceptible to cefoxitin antibiotics disk.

Livermore (10) used screening cefoxitin as a marker agent for the production of AmpC β-lactamases because this antibiotic is stable against the activity of multiple β-lactamase like TEM-1,-2, SHV-1 and ESBL but hydrolyzed by AmpC enzyme. Therefore, the resistance to cefoxitin may be due to the presence of AmpC enzyme or reduction in outer membrane permeability (37).

According to types of antibiotics resistance patterns, high rate of MDR 9/12 (75%) was detected, followed by PDR 2/12 (16.6%), while XDR was detected only in one isolate 1/12 (8.3%) (Figure 2).

Llaca-Diaz et al. (41) found antibiotic resistance, data detailing the MIC 50, MIC 90 and percentages of antimicrobial resistance for each of the ESKAPE pathogens, as well as for a few other frequent species, are presented. In general, a high prevalence of drug resistance was detected.

Conclusions:

Half rate of ESKAPE pathogens group in pediatric patients was recovered from burn unit. S. aureus was recovered with high rate among all ESKAPE pathogens group. However, no isolate belonged to E. faecium was recovered in this study. CoNS are the most prevalent pathogens causing late onset sepsis in neonates. MDR was found in high rate among ESKAPE pathogens group isolates. ESBLs were predominant among G-veof ESKAPE pathogens group local isolates. All isolates in ESKAPE pathogens group were AmpC β-Lactamase producers.

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References


