Epidemiology of Community Origin of major multidrug-resistant ESKAPE uropathogens in a paediatric population in South-East Gabon

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Abstract

Background: Urinary tract infections (UTIs) in children are very common. They are often associated with a high risk of sepsis and death. In addition, antibiotic resistance of UTI pathogens isolated from children is steadily increasing, especially against commonly used antibiotics.

The study's main objective was to examine the epidemiology of community origin and antibiotic sensitivity of major ESKAPE uropathogens in paediatric UTIs in South-East Gabon.

Methods: The study was conducted from January 2018 to December 2021 and involved 508 children aged 0-17 years. Identification of bacterial isolates was carried out using the Vitek-2 compact automated system and the antibiogram with the disk diffusion and microdilution methods according to the European Committee on Antimicrobial Susceptibility Testing recommendations.

Results: The prevalence of UTIs was 59%. *E. coli* and *K. pneumoniae* were the main ESKAPE involved in UTIs followed by *Enterococcus spp*. and *S.*aureus. The multidrug-resistant (MDR) phenotype was the most common. DTR-*E. coli*, CRE-*K. pneumoniae* and MDR-*K. pneumoniae* were associated with pyelonephritis. MRSA UTIs were frequent in symptomatic children. ESC-*E. coli* and MRSA were associated with recurrent UTIs while VRE and ESC-*E. coli* were associated with empirical treatment failures. MDR-*E. coli*, ESC-*E. coli*, MDR-*K. pneumoniae*, ESC-*K. pneumoniae*, UDR-*K. pneumoniae*, CRE-*K. pneumoniae* and XDR-*K. pneumoniae* were associated with rural paediatric populations.

Conclusion: This study describes the resistance phenotypes DTR, UDR and MAR index in Gabon. It showed a high prevalence of paediatric UTIs with high frequency of *E. coli*, *K. pneumoniae*, *Enterococcus spp*. and *S. aureus* with heterogeneous resistance profiles (MDR, XDR, DTR, ESC, CRE, MRSA and VRE).

Introduction

In human medicine, urinary tract infections (UTIs) are the second most common infection worldwide. It contributes significantly to morbidity and mortality in outpatient and inpatient settings representing approximately 20–60% of all infections [1, 2].

In a paediatric population, an estimated 7% of girls and 2% of boys will develop at least one UTI by age 6 [3]. Paediatric UTIs are common and often associated with a high risk of sepsis and death [4]. In most cases, the symptoms are nonspecific, especially in newborns and infants [5]. Recurrences are frequent (30–40% of cases), renal scarring is not uncommon and can lead to long-term high blood pressure and nephron reduction [6]. Infection is fostered by the presence of a functional or organic abnormality responsible for colonisation of the bladder, urinary stasis or reflux into the upper urinary tract [5]. Normal GI flora is usually the reservoir of bacteria found in urinary tract infections [7].

Overall, the common causative agents of UTIs are Gram-negative bacteria, mainly *Escherichia coli* (*E. coli*) followed by *Klebsiella pneumoniae* (*K. pneumoniae*) and *Proteus mirabilis*; while *Enterococcus faecalis* is the most common Gram-positive bacteria [8]. However, the epidemiology and distribution of uropathogenic species have shown strong geographical and temporal variations but also a relationship with the patient

populations studied [9]. An earlier study showed that the aetiology of UTIs has evolved significantly in hospital and community settings [10]. There is an increasing switch to "less common" micro-organisms with more pronounced roles including pathogens such as *Enterococcus faecalis* (E), *Staphylococcus aureus* (S), *Klebsiella pneumoniae* (K), *Acinetobacter baumannii* (A), *Pseudomonas aeruginosa* (P) and *Enterobacteriaceae* (E) known as *ESKAPE* group [10]. Through its overall induced mortality and economic impact, the *ESKAPE* group represents the greatest clinical challenge for antibiotic resistance surveillance and management interventions [11]. These bacteria are considered a priority by the World Health Organization (WHO) in the global monitoring of antibiotic resistance [12]. Indeed, *ESKAPE* pathogens frequently exhibit acquired resistance to a variety of antimicrobial agents, such as oxazolidinones, lipopeptides, macrolides, fluoroquinolones, tetracyclines, β-lactams (including carbapenems and combinations of beta-lactamase inhibitors) [13]. These acquired resistances result in the emergence of *ESKAPE* multidrug-resistance (MDR), extensive drug-resistance (XDR), pandrug-resistance (PDR), Extended-spectrum cephalosporin-resistance (ESC) and Carbapenem-resistant Enterobacteriales (CRE). In paediatrics, the situation is rather worrying because many antibiotic molecules available for adults (quinolones, fosfomycin, nitrofurantoin, mecilinam, etc.) are contraindicated in children or do not have a marketing authorisation or paediatric dosage form [5]. In addition, antibiotic resistance of UTI pathogens isolated from children is increasing, especially for commonly used antibiotics [14]. In South-East Gabon, a previous study on UTIs showed high frequency of resistance of bacteria to antibiotics in children under 5 years [15]. The choice to study *ESKAPE* pathogens isolated from paediatric UTIs is justified by the state of the Gabonese health system, which is confronted with both a glaring shortage of paediatricians and clinical microbiology laboratories.

The aim of this study was to determine the epidemiology and antibiotic sensitivity of major *ESKAPE* uropathogens in community-acquired paediatric urinary tract infections in Southeastern Gabon.

Methods

Design, study area and population

The study was conducted from January 2018 to December 2021. It involved all children aged 0 to 17 years, identified in the community as requesting a cyto-bacteriological examination of urine (CBEU) by the only microbiology laboratory in the city of Franceville, capital of the Haut-Ogooué province in the South-East of Gabon, bordering the Republic of Congo. During the study period a single paediatrician was practicing in the city.

Children included in the study were stratified into four paediatric populations: newborns and infants (0 to 2 years), early childhood (3 to 6 years), late childhood (7 to 12 years) and adolescence (13 to 17 years).

Inclusion Criteria

All non-hospitalised children of both sexes referred to the Microbiology Laboratory for a CBEU were eligible for inclusion in this study.

Sample Collection And Data Collection

For individual children, urine samples were collected as previously described [15]. For children who were not able to use the toilet alone, urine collection by 'clean capture' was preferred. Otherwise, urine was collected in a sterile adhesive collection bag with the help of parents, carers or a nurse.

The urine bottle was sealed, identified, indicating the time of collection and then transported to the laboratory at room temperature. The sociodemographic and clinical data of each patient was collected through a structured questionnaire.

Culture And Identification Of Bacterial Isolates

The bacterial culture consisted of aseptically inoculating ten microliters (10 µL) of total urine using a sterile single-use loop in the level 2 microbiological safety station. The inoculation was carried out systematically on Agar Media, CLED (Cystine-Lactose-Electrolyte-Déficient, bioMérieux, Marcy-l'Étoile, France), Mac Conkey (McC, bioMérieux, Marcy-l'Étoile, France) and COS (Columbia agar + 5% sheep blood, bioMérieux, Marcy-l'Étoile, France). Urine samples were inoculated within two (2) hours of collection to avoid contamination. The inoculated media were incubated aerobically in a bacteriological incubator at 35°C for 18 to 24 hours. According to Kass criteria, a number $\geq 10^5$ colony forming units (CFU)/mL was considered positive; a colony number $< 10^5$ CFU/mL or with more than two (2) types of bacterial colonies were considered contamination [16]. The bacterial count was done independently of the sex of the patient and of the pathogen isolated.

Presumptive identification of *ESKAPE* isolates was made after Gram stain, oxidase test to differentiate fermentative from non-fermentative Gram-negative bacilli, catalase test to discriminate genus *Staphylococcus* from genera *Streptococcus* and *Enterococcus.* Conventional biochemical tests (automated VITEK-2 system, bioMérieux, Marcy-l'Étoile, France) allowed the complete identification of bacterial genera, species and subspecies. The procedure for sample preparation and identification by VITEK-2 has been described in a previous study [17].

Antibiotic Sensitivity Test

The antibiotic sensitivity of *ESKAPE* isolates was determined by the diffusion disc method (Kirby-Bauer) on Mueller-Hinton (MH) agar (bioMérieux, Marcy-l'Étoile, France) according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [18]. Briefly, MH agars were seeded with a standardised suspension (0.5 McFarland) of each *ESKAPE* isolate from the 24-hour primary cultures. Antibiotic discs (Oxoid, Basingstoke Hampshire, UK) were firmly placed on the surface of the seeded plates. The culture media were then incubated at 35°C for 24 hours. The inhibition diameters around each antibiotic were interpreted according to EUCAST.

For antibiotics requiring microdilution methods, the minimum inhibitory concentrations (MIC) were measured on VITEK 2 Compact-AST (bioMérieux, Marcy-l'Étoile, France).

For Gram-negative isolates, the following antibiotic discs were used: Ampicillin, Amoxicillin-clavulanic acid, Piperacillin-tazobactam, Ticarcillin, Cefalotin, Cefoxitin, Cefotaxime, Ceftazidime, Cefepime, Ertapenem, Imipenem, Gentamicin, Tobramycin, Amikacin, Nalidixic acid, Ofloxacin, Ciprofloxacin, Nitrofurantoin and Trimethoprim-sulfamethoxazole.

The panel of antibiotics used for Gram-positive isolates was: Moxifloxacin, Erythromycin, Clindamycin, Quinupristin-dalfopristin, Linezolid, Vancomycin, Tetracycline, Tigecycline

, applicable to all gram-positive bacteria.

For *Enterococcus spp.*, Ampicillin and nitrofurantoin were also tested while the antibiotics Benzylpenicillin, Oxacillin, Gentamicin, Ciprofloxacin, Levofloxacin and Trimethoprim-sulfamethoxazole were additionally tested on *Staphylococcus aureus* isolates.

Classification Of Antibiotic Resistance Phenotypes

Resistance phenotypes have been classified into seven categories: Multi-drug Resistant (MDR), Extensive Drug Resistant (XDR), Pandrug-resistant (PDR), Usual Drug Resistance (UDR), Difficult-to-Treat Resistance (DTR), Extended-spectrum cephalosporin-resistant (ESC), Carbapenem-resistant Enterobacterales (CRE). These different categories of resistance have been defined in several previous studies [10, 19, 20].

Multiple antibiotic resistance (MAR) index was determined for each isolate using the formula MAR = n/N, where n represents the number of antibiotics to which the test isolate showed resistance and N represents the total number of antibiotics to which the test isolate has been evaluated [21]. The MAR index ranged from 0.0 to 1.0.

Statistical Analysis

All statistical analyses and graphs were performed with R software, version 4.0.2. The chi-square test was used to compare the prevalence of different phenotypes of each bacterium in the study population. In addition, the co-occurrence of resistance between different families of antibiotics (Beta-lactams, Quinolones, Aminoglycosides, Sulfonamides and Nitrofurans) was highlighted by a Venn diagram with the "Venn Diagram" package version 1.7.3. To determine the biological and demographic factors that may play an important role in the occurrence of pyelonephritis, a generalized linear binomial model was generated with the statistics package version 3.6.2 using the "glm" function and the choice of the best model, delta AIC = 0, was made using the dredge function of the MuMin package.

Results

Socio-clinical characteristics of study patients

A total of 608 urine samples was collected over a 4-year period, of which 100 were excluded from the study. Males represented 56% of the study population (289/508). The male/female sex ratio was 1.31. The patients'

mean age was 3.96 ± 4.67 years, and 57% (293/508) were newborns and infants. A large majority of patients were from urban areas (78%, 397/508), with an urban/rural ratio of 3.57.

Regarding the clinical signs observed in the latter, 43% (220/508) had fever while 42% (215/508) had a history of pre-emptive antibiotic therapy (Table 1).

Prevalence of urinary tract infections in community paediatrics

The overall prevalence of UTIs was 59% (304/508). UTIs were associated with age in the paediatric population. They were significantly more frequent in neonates and infants compared to other age group (78.2%; p <0.001) (Table 1). Boys and girls were similarly affected by UTIs (Table 1).

Regarding residence, UTIs were not significantly more prevalent in patients from urban areas than those from rural areas (62.2% vs 51.4%; p=0.05).

Non-febrile children were more susceptible to UTIs (98.6% vs 60.9; p<0.0001) (Table 1).

Bacteriological profile of UTIs

UTIs were significantly associated with uropathogens in the *ESKAPE* group compared to the non-*ESKAPE* group (89% vs 11%; p <0.0001). Among the *ESKAPE* pathogens, the *ESKAPE*-Gram- group was significantly more prevalent than the *ESKAPE*-Gram+ group (75% vs 14%; p<0.001) (Table 2). Within the *ESKAPE*-Gram- uropathogens, the predominant bacteria were *E. coli* (46%, 105/229) and *K. pneumoniae* (45%, 104/229).

Among the *ESKAPE*-Gram+ uropathogens, *Enterococcus spp.*, and *Staphylococcus aureus* accounted for 58% (25/43) and 42% (18/43), respectively (Table 2).

Frequency of resistance phenotypes of major uropathogenic strains.

Among *E. coli* and *K. pneumoniae* isolates, ESC (65%, 136/209), MDR (64%, 135/209), and UDR (52%, 108/209) resistance phenotypes were more frequent compared to other phenotypes (Table 3).

The frequency of MDR-*K. pneumoniae* strains was similar to that of MDR-*E. coli* (56% vs 44%; p=0.05), while DTR-*E. coli* was significantly more prevalent than DTR-*K. pneumoniae* (100% vs 0%, p=0.01). No statistically significant differences were observed when comparing the other phenotypes (Table 3). Furthermore, DTR-*E. coli* was associated with low back pain and haematuria while CRE-*E. coli*, MDR-*E. coli* and UDR-*E. coli* were associated with urinary burning, fevers/pollakiuria and abdominopelvic pain, respectively (Figure 1A).

Data also showed that ESC-*E. coli* was associated with recurrent infections and empirical treatment failures (Figure 1A). The distribution of resistance categories in *K. pneumoniae* showed that XDR-*K. pneumoniae* and CRE-*K. pneumoniae* were associated with male children presenting with fever and pollakiuria (Figure 1A).

For MDR-*K. pneumoniae*, ESC-*K. pneumoniae* and UDR-*K. pneumoniae*, these phenotypes were more frequently observed among girls in little childhood group (Figure 1A).

In general, the MDR-*E. coli*, ESC-*E. coli*, MDR-*K. pneumoniae*, ESC-*K. pneumoniae*, UDR-*K. pneumoniae*, CRE-*K. pneumoniae* and XDR-*K. pneumoniae* phenotypes were associated with the rural paediatric population (Figure 1A).

Distribution of resistance phenotypes in *S. aureus* and *Enterococcus spp*.

Among *S. aureus* and *Enterococcus spp*. isolates, MDR (86%, 37/43) and UDR (81%, 35/43) phenotypes were the most frequent. Among MDR strains, vancomycin-sensitive *Enterococci* (VSE) (51%,19/37) were significantly more frequent than the other resistance phenotypes ($p<0.0001$). Similarly, the UDR phenotype was significantly associated with vancomycin-sensitive *Enterococci* (VSE) ($p= 0.01$) (Table 4).

UDR and XDR phenotypes were associated with MSSA and MRSA, respectively (Figure 1B).

MSSA UTIs were associated with symptomatic children (abdominal-pelvic pain). MRSA was also associated with recurrent UTIs and male children (Figure 1B).

Vancomycin-resistant *Enterococci* (VRE) were more observed in children of rural areas who had been treated with antibiotic therapy (Figure 1B).

Role of biological and demographic factors in the occurrence of pyelonephritis.

The results of the generalized linear binomial model (glm) showed that of all the socio-clinical-biological factors listed, only CRE-*K. pneumoniae* and MDR-*K. pneumoniae* were significantly associated with pyelonephritis (Table 5).

Distribution of antibiotic resistance among major uropathogens.

E. coli and *K. pneumoniae* isolates were associated with resistance to Ticarcillin, Piperacillin-Tazobactam, Cefepime, Ertapenem, Gentamicin, Tobramycin, Ciprofloxacin, Ofloxacin, Nitrofurantoin and Trimethoprim-sulfamethoxazole in children in rural areas (Figure 1A).

Overall, resistance to the above antibiotics was linked to MDR-*E. coli*, MDR-*K. pneumoniae*, ESC-*K. pneumoniae*, CRE-*K. pneumoniae*, XDR- *K. pneumoniae* and UDR-*K. pneumoniae* phenotypes (Figure 1A).

Resistance to Cefoxitin, Ceftazidime, Imipenem and Nalidixic acid was associated with *E. coli* strains isolated from children in urban areas. Similarly, Cefotaxime resistance was associated with empirical antibiotic failure and recurrent UTIs (Figure 1A).

S. aureus and *Enterococcus spp*, resistance to Gentamicin, Erythromycin, Clindamycin, Quinupristin-dalfopristin, Oxacillin was associated with Vancomycin-Resistant Enterococci, while resistance to Benzylpenicillin, Levofloxacin, Ciprofloxacin, and Tigecycline was associated with MRSA UTIs (Figure 1B). Figure 1B also shows that Tigecycline resistance was associated with recurrent UTIs.

Venn diagram (Figure 2) shows the distribution and relationship between resistance of different families of antibiotics tested with *E. coli* and *K. pneumoniae* isolates.

Overall, the highest rate of resistance was observed within the β-lactam family with 88% (184/209) followed by sulfonamides (65%, 137/209) and quinolones (50%, 106 /209) against 1% (3/209) observed for the family of nitrofurans (Figure 2).

Venn diagram also reported that 11% (23/209), 0.95% (2/209) and 1.43% (3/209) of *E. coli* and *K. pneumoniae* isolates showed monoresistance to β-lactams, Aminoglycosides and sulfonamides (Figure 2).

The co-occurrence of resistance to two antibiotic families was predominantly observed with β-lactam/sulfonamide combination (63%, 132/209) (Figure 2).

The co-occurrence of resistance to three antibiotic families was highest with the combination β-lactam-sulfonamide-aminoglycoside (12.9%, 27/209) while β-lactam-aminoglycoside-sulfonamide-quinolone combination showed the highest co-occurrence with four antibiotic families (27%, 57/209). Only one isolate showed resistance to all antibiotic families tested (Figure 2).

Multiple antibiotic resistance (MAR) index of *E. coli* and *Klebsiella spp.*

The MAR index results showed that more than half of *E. coli* and *K. pneumoniae* isolates had resistance to at least 5 antibiotics (MAR index ≥ 0.26) while only one isolate showed resistance to 17 antibiotics (MAR index = 0.89) (Table 6).

Discussion

Several studies in resource-limited countries suggested that major pathogens found in urinary tract infections are often resistant to standard antibiotics [22], especially those isolated from UTIs in children [10]. The aim of this study was to examine the epidemiology and antibiotic susceptibility of major *ESKAPE* uropathogens in community-acquired paediatric UTIs in South-East Gabon.

The prevalence of urinary tract infections was 59%. They were significantly more frequent in neonates and infants (Table 1), corroborating the findings of Hay et al, who found a 46.6% rate of UTIs in children under 5 years [16].

E. coli and K. pneumoniae were the main uropathogenic *ESKAPE*s isolated in this study, with 35% and 34%, respectively, followed by *Enterococcus spp*. and *S. aureus* (Table 2). These results corroborate those of previous studies conducted in Africa [23–25]. Many virulence and fitness factors confer advantages to uropathogenic *E. coli* (UPEC) and *K. pneumoniae* (UPKP) in the host urinary tract. UPEC usually has a superficial viruloma and a secretome that contribute to its virulence and survival [26].

We describe the DTR and UDR resistance phenotypes as well as MAR index in Gabon (Table 3&6). These resistance phenotypes are now preferred to MDR, XDR and PDR phenotypes.

Indeed, MDR, XDR and PDR resistance phenotypes make no distinction between the strengths and weaknesses of each antibiotic: antibiotics with higher efficacy and lower toxicity are considered the same as those with lower efficacy and higher toxicity [27, 28]. Characterization of DTR, UDR, ESC, and CRE phenotypes

facilitates resistance monitoring for clinicians (Tables 3 & 4), as well as the improvement of empirical and targeted treatment regimens [27].

Among the major uropathogens, MDR resistance phenotype was the most common (Table 3). Similar rates of MDR uropathogenic strains have been previously described in Gabon [15]. However, a lower rate of MDR isolates was reported by Dikoumba et al [29]. The differences observed could be explained by the origin of the samples used in each study.

Early childhood girls were more susceptible to UTIs caused by MDR-*K. pneumoniae* and ESC-*K. pneumoniae* while XDR-*K. pneumoniae* and CRE-*K. pneumoniae* were more frequently found in male children (Fig. 1A). Male gender has already been described as a likely risk factor in the occurrence of MDR UTIs [20]. In the previously cited study, the authors showed that female gender was a protective factor in the occurrence of antibiotic-resistant UTIs, which does not support the results of the present study. The observation of multidrug-resistant phenotypes in male children could be a risk factor both for the failure of empirical treatment and for the aggravation of clinical forms. Indeed, male urinary tract infections present a high risk of complications because of the frequency of anatomical or functional abnormalities associated with them. [30].

DTR-*E. coli*, CRE-*K. pneumoniae* and MDR-*K. pneumoniae* were associated with pyelonephritis (Table 5, Fig. 1A). ESC-*E. coli* and MRSA were associated with recurrent UTIs while VRE and ESC-*E. coli* were associated with empirical treatment failures (Fig. 1A&B). Although the relationship between virulence and resistance appears to be antagonistic [31], authors have shown that one of the *E. coli* clones that globally disseminates extended-spectrum β-lactamases and NDM-1 carbapenemases, with few classical virulence factors, was virulent in a mouse model of sepsis [32, 33]. Thus, strains with virulence and resistance capabilities emerge as antibiotic selection pressure increases [34]. Other authors have provided epidemiological evidence that resistance and virulence phenotypes are linked in *E. coli* isolates of community origin [35]. Resistance phenotypes observed in this study strongly impact optimal care of community-based IVUs often treated by empirical antibiotherapy [36].

The presence of MDR, XDR, CRE, ESC, DTR, MRSA and VRE phenotypes may force clinicians to use antibiotics with less beneficial or limited pharmacological properties such as aminoglycosides (nephrotoxic, ototoxic and neurotoxic) and colistin (nephrotoxic and neurotoxic) [37].

In general, MDR-*E. coli*, ESC-*E. coli*, MDR-*K. pneumoniae*, ESC-*K. pneumoniae*, CRE-*K. pneumoniae* and XDR-*K. pneumoniae* phenotypes were more common in children from rural areas (Fig. 1A). UTIs are quite common in community settings. However, many patients refuse to seek medical attention because of the social stigma associated with UTIs in rural areas. In these areas, the lack of paediatricians also leads to long queues during medical consultations, discouraging many patients who opt for self-medication, largely responsible for the selection of resistant strains. Also, the lack of education and financial resources are two additional factors favouring the emergence and dissemination of bacterial resistance in rural UTIs.

In this study, the frequency of DTR, ESC, CRE, MDR, MRSA and ERV phenotypes was not negligible in paediatric UTIs. However, the high presence of the UDR phenotype (more than 50%) in major uropathogens suggests that these infections remain treatable with standard antibiotics.

Conclusion

This study describes for the first time the resistance phenotypes DTR, UDR and MAR index in Gabon. The prevalence of UTIs was high with a strong involvement of the uropathogens *E. coli*, *K. pneumoniae*, *Enterococcus spp*. and *S. aureus*. Neonates and infants were predominantly affected by UTIs. The study also showed that the majority of paediatric UTIs remain susceptible to standard antibiotic therapy. However, the presence of MDR, XDR, DTR, ESC, CRE, MRSA and VRE phenotypes in the paediatric population should alert health authorities to the need to set up a national surveillance system for antimicrobial resistance in Gabon.

Abbreviations

ESKAPE, *Enterococcus faecalis, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas, Enterobacteriaceae* ; IVUs Infection des voies urinaires ; MDR, Multidrug resistance ; XDR, Extensive drug resistance ; PDR, Pandrug-resistance ; DTR, Difficult to Treat Resistance ; UDR, Usual drug resistance ; ESC, Extended-spectrum cephalosporin-resistant ; CRE, Carbapenem-resistant *Enterobacterales* ; MAR, multiple antibiotic resistance ; SARM, Methicillin-resistant *Staphylococcus aureus*; SASM, Methicillin-sensitive *Staphylococcus aureus*; VRE, Vancomycin-resistant *Enterococci*; VSE, Vancomycin-sensitive *Enterococci*, UPEC, Uropathogenic-*E. coli* ; UPKP, Uropathogenic-*K. pneumoniae*; CFU, Colony-forming units

Declarations

Ethics approval and consent to participate

For all children, informed and written consent was obtained from their parents or legal guardians prior to inclusion in the study.

The research license for this study was obtained from the Scientific Commission on Research Authorizations of the National Centre of Scientific and Technological Research (CENAREST) (permit 7 no. AR0033/17/MESRSFC/CENAREST/CG/CST/CSAR, dated 4 July 2017). This study was conducted in accordance with the Declaration of Helsinki.

Consent for publication

All authors have read the manuscript and consent to publish.

Availability of data and materials

Data and materials supporting the conclusions of this study will be made available on request to the corresponding author.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YMN conceived of the study and participated in study design, execution, acquisition, interpretation of data, drafting, revising and critically reviewing the article. RO participated in interpretation of data, drafting, revising and critically reviewing the article. NMLP performed the statistical analysis. MB participated in statistical analysis. CB performed conception, study design, coordination, drafting, revising and critically reviewing the article. All authors read and approved the final manuscript.

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Tables

Table 1. Distribution of UTIs according to patients' socio-clinical parameters.

Characteristics	Total number (n= 508)	UTIs (n=304)	Percentage (%)	p-value
Pediatric population				
Neonates and infants	293	229	78.2	<0.0001
Early childhood	99	37	37.4	
Late childhood	59	14	23.7	
Adolescents	57	24	42.1	
Sex				
Male	289	168	58.1	NS
Female	219	136	62.1	
Residence				
Urban	397	247	62.2	0.05
Rural	111	57	51.4	
Symptoms				
Urinary signs	215	98	45.6	
Voiding burns	68	39	57.3	
Pollakiuria	40	21	52.5	
Abdomino-pelvic pain	57	18	31.6	NS
Hematuria	8	3	37.5	
Low back pain	33	13	39.4	
Incontinence	9	4	44.4	
Non-urinary signs	293	206	70.3	
Fever	220	134	60.9	
Others	73	72	98.6	<0.0001
History of antibiotic therapy				
Probabilistic ATB	215	111	51.6	0.001

Non- Probabilistic ATB	293	193	65.9

Table 2: Uropathogens isolated from children UTIs.

Urinary tract infections		
	Total number (n=304)	**Percentage (%)**
Uropathogens		
ESKAPE-Gram-	**229**	**75**
Escherichia coli	105	35
Klebsiella pneumoniae	104	34
Pseudomonas aeruginosa	1	0.3
Acinetobacter baumannii	2	0.7
Citrobacter-Enterobacter-Serratia (CES)	11	4
Proteus mirabilis	6	1
ESKAPE-Gram+	**43**	**14**
Staphylococcus aureus	18	6
Enterococcus spp.	25	8
Non-ESKAPE	**32**	**11**
Morganella morganii ssp morganii	2	0.7
Staphylococcus coagulase negative	23	8
Streptococcus spp.	2	0.7
Micrococcus lentus	1	0.3
Candida albicans	4	1.3

Table 3: Prevalence of major ESKAPE-Gram- uropathogens according to resistance categories.

Resistance Categories										
	MDR (n= 135)	**Non-MDR (n= 74)**	**XDR (n= 9)**	**Non-XDR (n= 200)**	**ESC (n= 136)**	**Non-ESC (n= 73)**	**CRE (n= 11)**	**Non-CRE (n= 198)**	**UDR (n= 108)**	**DTR (n= 5)**
E. coli	59 (44%)	46 (62%)	6 (67%)	99 (49%)	62 (46%)	43 (59%)	3 (27%)	102 (52%)	60 (56%)	5 (100%)
K. pneumoniae	76 (56%)	28 (38%)	3 (33%)	101 (51%)	74 (54%)	30 (41%)	8 (73%)	96 (48%)	48 (44%)	0 (0%)
Chi-squared	3.8	7.8	0.89	0.01	1.8	3.9	2.9	0.25	2.2	6.4
df	1	1	1	1	1	1	1	1	1	1
P-value	**0.05**	**0.005**	NS	NS	NS	**0.04**	NS	NS	NS	**0.01**

Abbreviation : NS, not significant.

Table 4: Prevalence of major ESKAPE-Gram+ uropathogens in resistance categories.

Resistance Categories			
	MDR (n= 37)	**XDR (n= 2)**	**UDR (n= 35)**
MRSA	10 (27%)	1 (50%)	9 (26%)
MSSA	3 (8%)	0 (0%)	8 (23%)
VRE	5 (14%)	1 (50%)	3 (8%)
VSE	19 (51%)	0 (0%)	15 (43%)
Chi-squared	22.0	2.7	11.1
df	3	3	3
P-value	**<0.0001**	NS	**0.01**

Abbreviation : NS, not significant

Table 5: Biological and demographic factors that could play an important role in the occurrence of pyelonephritis

	Fixed effects					
Model No. (rank)	**Intercept**	***Klebsiella pneumoniae*-CRE**	***Klebsiella pneumoniae*-MDR**	**Df**	**ΔAIC**	**Akaike weight**
4 (1)	-0.3385	**+**	**+**	**3**	**0**	0.532
3 (2)	-0.3385		+	2	1.29	0.279

Table 6: Multiple antibiotic resistance (MAR) index of *E. coli* and *K. pneumoniae*

MAR index*	Isolate frequency (%)
0.00	24.1
0.05	4.0
0.10	2.0
0.15	5.0
0.21	5.0
0.26	11.0
0.31	6.0
0.36	4.0
0.42	10.0
0.47	7.0
0.52	6.0
0.57	4.0
0.63	2.0
0.68	5.0
0.73	2.0
0.78	2.0
0.89	**0.9**

Figures

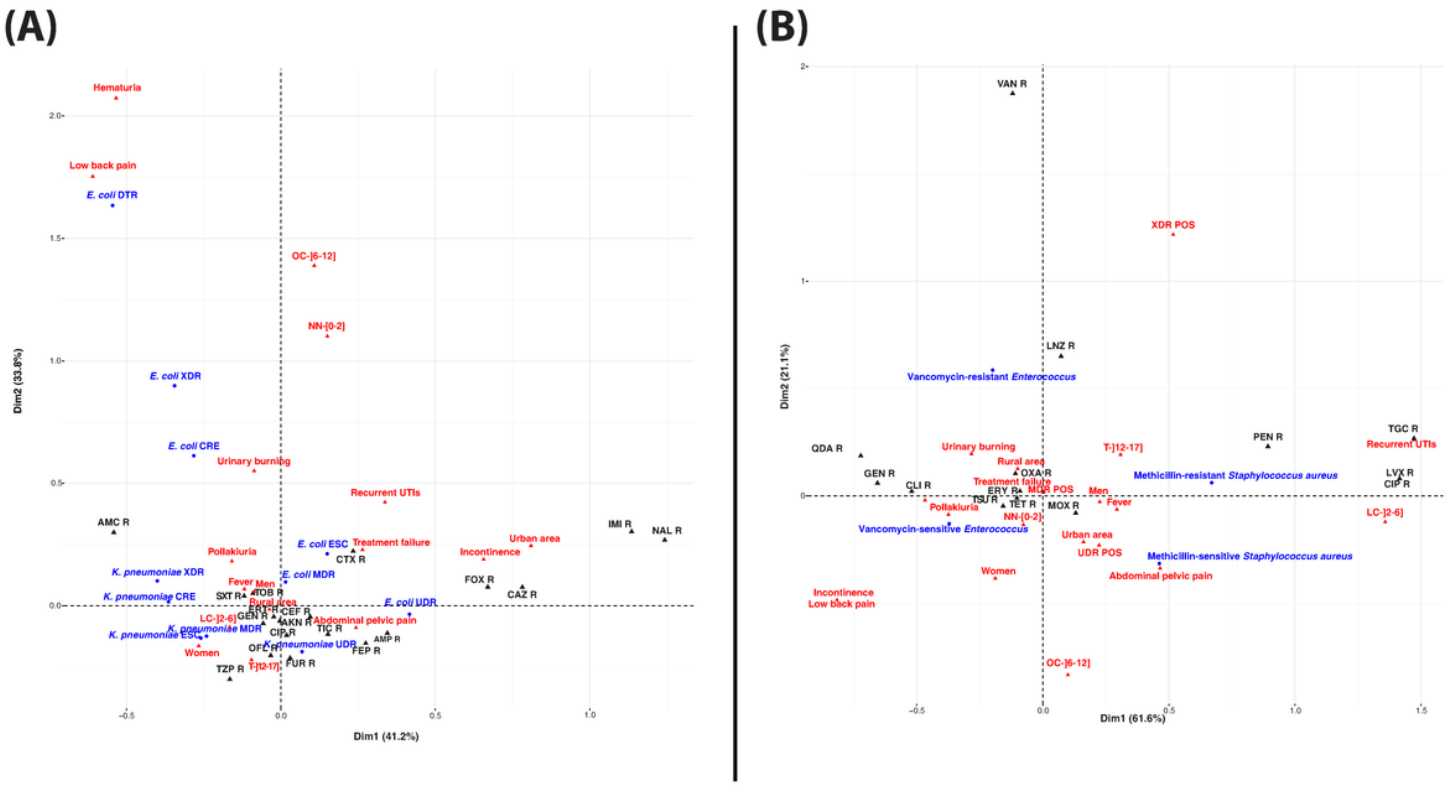

Figure 1

Distribution of major *ESKAPE* resistance phenotypes according to socio-clinical and biological parameters.

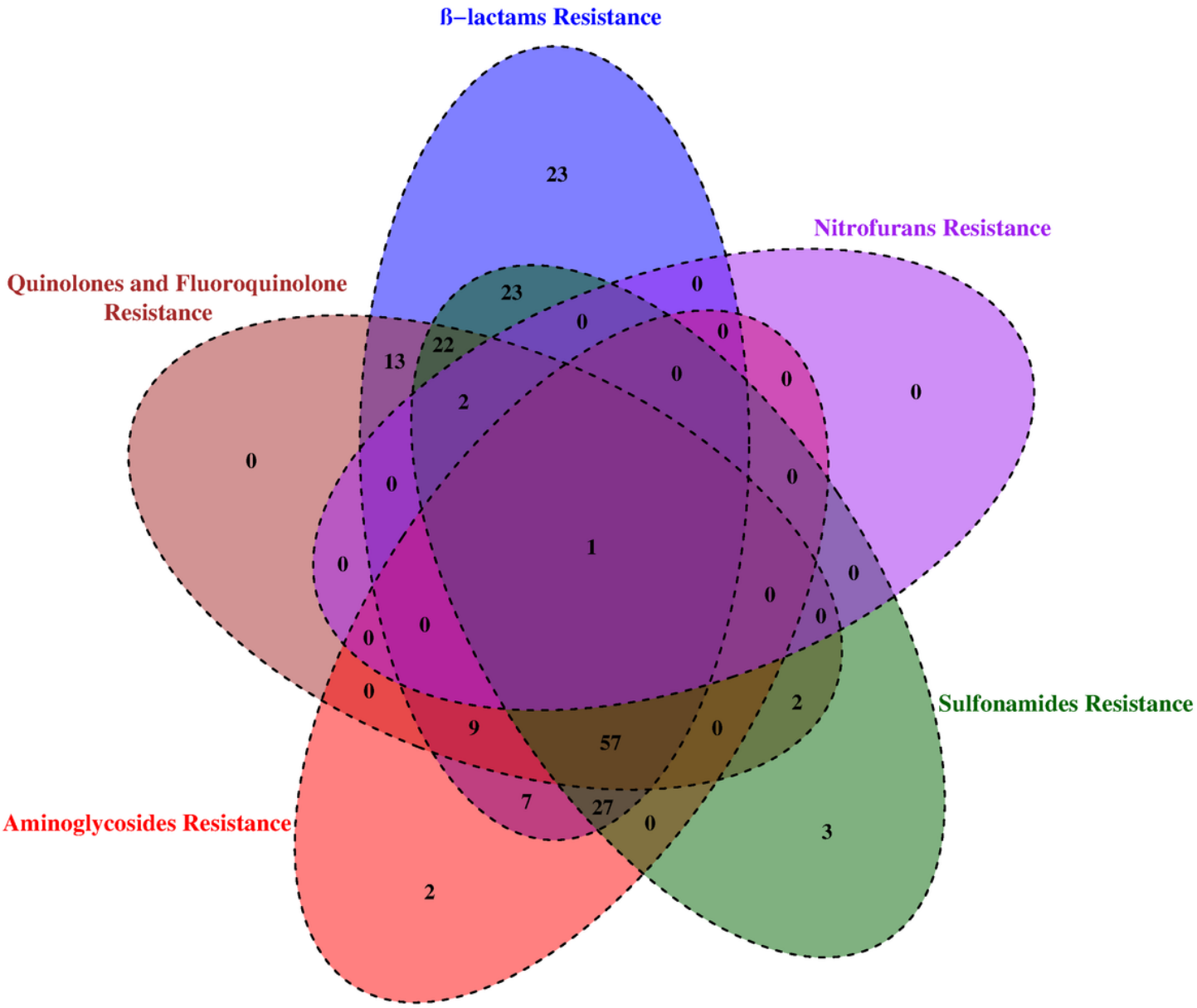

Figure 2

Co-occurrence of resistance to antibiotic families in *E. coli* and *K. pneumoniae* isolates.