

Multidrug-resistant *Klebsiella* sp. one of the main etiological agents of urinary and respiratory tract infections in infants at southeastern Mexico

Klebsiella sp. multidrogorresistente, uno de los agentes etiológicos principales de infecciones de los tractos urinario y respiratorio en infantes del sureste de México

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Abstract

Goals. The purpose of the work was to determine the *Klebsiella* species affecting hospitalized infants at southeastern Mexico, their antimicrobial susceptibility profile, the resistance phenotype, and the presence of ESBL enzymes. **Methodology.** From 2015 to 2020, a total of 794 records of bacteriological cultures positive to *Klebsiella* spp. were included. The frequencies of bacterial species, the biological material from which they were isolated, the age groups affected, the antimicrobial susceptibility profiles, the resistance phenotype, and the detection of ESBL enzymes were obtained. Data were analyzed using descriptive statistics and bivariate analysis, with a significance level of $p \leq 0.05$. **Contribution.** *K. pneumoniae subsp. pneumoniae* was the most frequent bacteria isolated from urine, blood and bronchial aspirate samples. Most of them were resistant to beta-lactams and cephalosporins, in addition to exhibiting the MDR phenotype associated with the presence of ESBL enzymes. The susceptibility of the strains to meropenem and ertapenem indicates that carbapenems are the treatment alternative to combat these nosocomial infections in infants at southeastern Mexico.

Resumen

Objetivos. Determinar las especies de *Klebsiella* que afectan a infantes que acuden a un hospital del sureste mexicano, su perfil de susceptibilidad a los antimicrobianos, el fenotipo de resistencia y la presencia de enzimas BLEE. **Metodología.** Del 2015 al 2020, se compilieron los resultados de 794 cultivos bacteriológicos positivos a *Klebsiella* sp.. Se obtuvieron las frecuencias de especies bacterianas, el material biológico del que se recuperaron, los grupos de edad afectados, los perfiles de susceptibilidad antimicrobiana, el fenotipo de resistencia y la detección de las enzimas BLEE. Los datos se analizaron con estadística descriptiva y análisis bivariado, con un nivel de significancia de $p \leq 0.05$. **Contribución.** Se determinó que *K. pneumoniae subsp. pneumoniae* fue la más frecuente aislada en muestras de orina, sangre y aspirado bronquial. La mayor parte de los aislados fueron resistentes a los beta lactámicos y cefalosporinas, además de exhibir el fenotipo MDR asociado a la presencia de enzimas BLEE. La susceptibilidad de las cepas a meropenem y ertapenem indica que los carbapenémicos son la alternativa de tratamiento para combatir estas infecciones nosocomiales en infantes del sureste mexicano.

Klebsiella, Nosocomial, Susceptibility, Southeastern

Klebsiella, Nosocomial, Susceptibilidad, Sureste

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Introduction

Antimicrobial drugs are one of the vital tools in therapy, however, their uncontrolled use favours the emergence of bacteria resistant to them, or such prokaryotes acquire this phenotype by genetic mechanisms (Nercelles et al., 2000). The group of recognised nosocomial bacteria with high antimicrobial resistance are *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter*, which make up the group known as ESKAPE (acronym formed by the first letter of each genus) (Rice, 2008). Of these, *K. pneumoniae* is the main reservoir of infections at the hospital level, favoured by its ability to exhibit antimicrobial resistance to at least three different categories of antibiotics (multidrug-resistant or MDR) (Navon-Venezia et al., 2017). The Mexican drug resistance research and surveillance group (INFIVAR) has identified *Acinetobacter* sp. *Klebsiella* sp. and *E. coli* as the most frequent MDR bacteria; in addition, *K. pneumoniae* and *E. coli* strains exhibit a high presence of genes encoding for beta-lactamases, mainly CTX-M-15 (Garza-González et al., 2019, 2021).

This paper shows the resistance profile of *Klebsiella* sp. strains isolated from infants in a hospital in Chiapas, the state with the highest poverty in Mexico (Consejo Nacional de Evaluación de La Política de Desarrollo Social (CONEVAL). Measuring Poverty 2008-2016, 2017). These data provide information on this nosocomial bacterium that affects this vulnerable population, which contributes to the therapeutic approach that can be used to combat this aetiological agent in paediatric infections.

Material and methods

The study was descriptive and retrospective, based on records from 2015 to 2020 captured in the WHONET© platform, of *Klebsiella* genus isolates from outpatients and inpatients treated at the Hospital de Especialidades Pediátricas de Tuxtla Gutiérrez, Chiapas. Antimicrobial susceptibility testing and BLEE enzyme detection were processed in the Vitek® 2 Compact kit, following the manufacturer's instructions.

The continuous variable was the age of the patients, while the categorical variables were age groups, sex, hospital area, type of biological sample, date of collection, *Klebsiella* species isolated, antimicrobial susceptibility profile according to the established cut-off values (CLSI, 2021), resistance phenotype (multidrug-resistant -MDR-, extensively drug-resistant -XDR- and pandrug-resistant -PDR- (Magiorakos et al, 2012) and the presence of BLEE enzymes. Detection of these enzymes was corroborated by the double diffusion test with discs on Müller Hinton agar, placing a disc of cefotaxime (CTX, 30 µg) in the centre with that of the beta-lactamase inhibitor clavulanic acid (CLA, 10 µg) and at 25 mm the discs of the monobactam Azteronom (ATM, 30 µg) and the cephalosporins ceftazidime (CAZ, 30 µg), ceftriaxone (CRO, 30 µg) and cefepime (FEP, 30 µg) were placed; the occurrence of synergy between the inhibitor with the cephalosporins (fishtail or egg effect) constitutes a positive BLEE test, while a negative test is recorded with the occurrence of inhibition halos with CLA >=18 mm, ATM >=21 mm, CAZ >=21 mm, CRO >=23 mm and FEP >=25 mm (CLSI, 2021). Descriptive statistical analyses and Pearson's correlation between the proportion of *Klebsiella* strains and year of isolation were performed; categorical variables were examined with Fisher's exact test, with a significance level of p≤0.05; SigmaPlot software (version 12.0) was used for the analyses.

Results

In the five-year period, a total of 794 patients were registered, of which the majority were male (57.9%) and the rest female (42.1%). *K. pneumoniae* subsp. *pneumoniae* was the most frequent species (89.9%), followed by *K. pneumoniae*, *K. oxytoca* and *K. pneumoniae* ozaenae (5.7, 3.7 and 0.8%, respectively). Most species were recovered from urine, blood and bronchial aspirate (30.1, 23.6 and 16.9%, respectively), while, of the fomites, the majority were recovered from catheter tips (11.2%) (Table 1).

Source of insulation	Species of <i>Klebsiella</i>			
	<i>K. pneumoniae</i> subsp. <i>pneumoniae</i>	<i>K. pneumoniae</i>	<i>K. oxytoca</i>	<i>K. pneumoniae</i> subsp. <i>ozaenae</i>
Urine N(%)	215 (30.1)	17 (37.8)	6 (20.7)	0 (0.0)
Blood N (%)	167 (23.4)	7 (15.6)	11 (37.9)	2 (33.3)
Bronchial aspirate N (%)	124 (17.4)	6 (13.3)	2 (6.9)	2 (33.3)
Wound N (%)	81 (11.3)	5 (11.1)	6 (20.7)	1 (16.7)
Faeces	9 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)
Abscess	8 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)
Vaginal/vulvar exudate	6 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)
Sputum	4 (0.6)	1 (2.2)	0 (0.0)	0 (0.0)
Water	4 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)
Bronchoalveolar lavage	3 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)
Pleural dialysis fluid	3 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)
Pleural fluid	2 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)
Tissues	2 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)
Otic secretion	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Anal swab	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Catheter tip	76 (10.6)	8 (17.58)	4 (13.8)	1 (16.7)
Catheter exit site	7 (1.0)	1 (2.2)	0 (0.0)	0 (0.0)
Penrose	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)

Table 1 Distribution of *Klebsiella* sp. species by source of isolation in Chiapas, Mexico

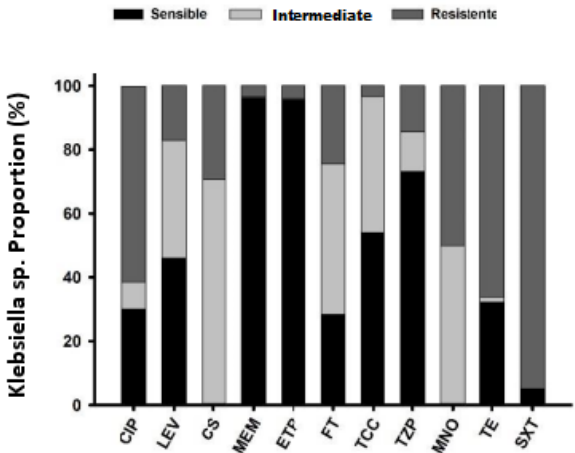
Similarly, a study in hospitals in North America and Europe showed that of the enterobacteria isolated from patients with urinary tract infection (UTI), *E. coli* was the most frequent (61.5%), followed by *Klebsiella* sp and *P. mirabilis* (19.5 and 6.6%, respectively) (Hoban et al., 2012). Boys, compared to girls, were more affected by *Klebsiella* species (57.9 vs. 42.1%, respectively), however, there were no significant differences (p=0.96). Significantly, almost half of the children under 5 years of age were more affected compared to the other age groups (Table 2); this could be due to the underdeveloped immune system in this group of infants.

Species of <i>Klebsiella</i>	Age groups (years)			P
	<5 N (%)	5-9 N (%)	10-19 N (%)	
<i>K. pneumoniae</i> sp. <i>pneumoniae</i>	341 (47.8)	195 (27.3)	178 (24.9)	<0.001
<i>K. pneumoniae</i>	5 (11.1)	26 (57.8)	14 (31.1)	
<i>K. oxytoca</i>	9 (31.0)	13 (44.8)	7 (24.1)	
<i>K. pneumoniae</i> <i>ozaenae</i>	4 (66.7)	0 (0.0)	2 (33.3)	

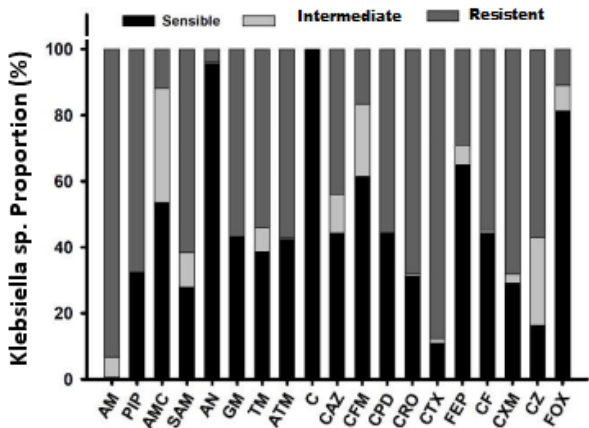
Table 2 *Klebsiella* sp. species by age group in Chiapas, Mexico

Similarly, a study in hospitalised Chinese infants revealed a higher proportion of *K. pneumoniae* strains isolated in the neonatal ward (29%) compared to the paediatric care unit (9%) (Hou et al., 2022).

No association between species isolated and year of isolation was observed (R2=0.03; p=0.73). The antimicrobial resistance profile of *Klebsiella* sp. revealed that most were resistant to sulfamethoxazole (94.9%), followed by ampicillin and cefotaxime (93.3 and 87.7%, respectively; figs. 1A and 1B).



Graph 1A Antimicrobial susceptibility profile of *Klebsiella* sp. strains isolated in Chiapas, Mexico. Fluoroquinolones (ciprofloxacin -CIP- and levofloxacin -LEV), polymyxins (colistin -CS-), carbapenemics (meropenem -MEM- and ertapenem -ETP-), nitrofurans (nitrofurantoin -FT-), extended-spectrum penicillins (ticarcillin/clavulanic acid -TCC- and piperacillin/tazobactam -TZP-), tetracyclines (minocycline -MNO- and tetracycline -TE-) and folate inhibitors (sulphamethoxazole -SXT-).



Graph 1B. Antimicrobial susceptibility profile of *Klebsiella* sp. strains isolated in Chiapas, Mexico. beta-lactams (ampicillin -AM-, piperacillin -PIP-, amoxicillin/clavulanic acid -AMC- and ampicillin/sulbactam -SAM-), aminoglycosides (amikacin -AN-, gentamicin -GM- and tobramycin -TM-), monobactams (aztreonam -ATM-), phenicols (chloramphenicol -C-), 3rd and 4th generation cephalosporins (ceftazidime -CAZ-, cefixime -CFM-, cefpodoxime -CPD-, ceftriaxone -CRO-, cefotaxime -CTX-, ceftazidime -FEP-) and 1st and 2nd generation cephalosporins (cephalothin -CF-, cefuroxime -CXM-, cefazolin -CZ- and cefoxitin -FOX-).

The high resistance to cefotaxime reported here may be due to the fact that the majority of *K. pneumoniae* isolates in Mexico (91.9%) possess the blaCTX-M gene, which encodes a class A beta-lactamase capable of hydrolysing that 3rd generation cephalosporin (Garza-González et al., 2021). Similar to what is shown here, other studies have revealed that more than half of *K. pneumoniae* strains isolated from the urinary and respiratory tract are resistant to cephalosporins (Haeili et al., 2013; Iqra et al., 2014). According to the antimicrobial resistance profiles, most strains were of the multidrug-resistant (MDR) phenotype (70.4%); no XDR or PDR phenotypes were detected. The majority of MDR strains exhibited the BLEE enzyme (Fig. 1), thus both characteristics were significantly associated (Table 3).

Antimicrobial resistance phenotype of <i>Klebsiella</i> sp.	Enzyme BLEE N (%)		
	Positive	Negative	P
MDR	479 (85.7)	80 (14.3)	<0.001
No MDR	50 (21.3)	185 (78.7)	

Table 3 Relationship between BLEE enzyme and antibacterial resistance phenotype in *Klebsiella* spp. from Chiapas, Mexico

This trend was similar in patients with UTI in a hospital in Monterrey, Mexico, where it was revealed that more than half of *K. pneumoniae* and *E. coli* isolates (59.6 and 59.4%, respectively) exhibited BLEE (Villalobos-Ayala et al., 2017). In contrast, all isolates in this study were susceptible to chloramphenicol, followed by meropenem (96.1%), amikacin (95.8%) and ertapenem (95.6%). To combat extended-spectrum beta-lactamase-producing enterobacteria, carbapenem antibiotics have represented a treatment option because of their stability against attack by these bacterial enzymes. (DeRyke et al., 2007).



Figure 1 Detection of BLEE in *Klebsiella* sp. by double diffusion test. Left: Positive BLEE test in a *Klebsiella* sp. strain with MDR phenotype, showing synergy between the CTX/CLA disc and cephalosporin discs (egg effect); right, Negative BLEE test in a non-MDR strain, exhibiting sensitivity to all discs.

Conclusions

The present study revealed that *K. pneumoniae* subsp. *pneumoniae* was the most frequent bacterium in urine samples, followed by blood and bronchial aspirate, with infants under five years of age being the most susceptible to these nosocomial infections. Most isolates were resistant to beta lactams and cephalosporins, with 70% of them having an MDR phenotype, and this characteristic was associated with the presence of BLEE enzymes. The susceptibility of most strains to meropenem and ertapenem indicates that treatment of infants with these carbapenemics should be the alternative to combat these nosocomial infections in southeastern Mexico.

Annexes

The database is available in the Mendeley repository (GUTIERREZ-JIMENEZ, JAVIER; Farrera-Ulloa, Isaura; Feliciano-Guzmán, José Manuel; López- Sántiz, José Raúl (2023), "Multidrug-resistant *Klebsiella* sp. is the main etiological agent of urinary and respiratory tracts infections in infants at southeastern Mexico", Mendeley Data, V1, doi: 10.17632/zjtbmgb6h4.1).

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