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ORIGINAL ARTICLE

Colonization by Multidrug Resistant Gram-Negative Bacilli: is it a matter for hospitalized children?

Colonização por bacilos Gram-negativos multirresistentes: isso é um problema para as crianças hospitalizadas?

Colonización por bacilos gram-negativos resistentes a múltiples fármacos: ¿es un problema para niños hospitalizados?

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ABSTRACT

Background and Objective: The colonization of skin and mucosa by multidrug resistant gram-negative bacilli (MR GNB) in hospitalized patients has received increasing attention. **Methods:** We evaluated the colonization by MR GNB to determine the MR GNB antimicrobial susceptibility profile and resistant genes in a paediatric population. A case-control study was carried out, patients considered colonized by MR GNB and patients non-colonized by MR GNB were evaluated. The variables that were compared between the groups were infection, central venous catheter, lung mechanical ventilation, long-term urinary catheter, and outcome. The sensitivity of the bacteria to the antimicrobial agents was analysed by the disc-diffusion technique, and the genetic diversity of the isolates was analysed by enterobacterial repetitive intergenic consensus sequence-polymerase chain reaction (ERIC-PCR). **Results:** Patients with infection had 4 times greater odds of being colonized by MR GNB than non-infected patients. Among children colonized by MR GNB, the presence of an invasive procedure increased the odds of infection by 15 times. In children who were not colonized by MR GNB, the presence of an invasive procedure increased the odds of infection by 4 times. Among the 20 strains of MR GNB analysed, lower resistance was found for carbapenem and amikacin. The most common resistance mechanism was the production of CTXM1 enzymes, followed by CTXM15 and SHV. **Conclusions:** Colonization by MR GNB was associated with infection in paediatric patients; therefore, measures should be taken to prevent colonization by MR GNB. Consequently, knowing the state of bacterial colonization is still important in pediatric wards.

Keywords: *Enterobacteriaceae; Drug resistance, microbial; Cross infection; Infection Control.*

RESUMO

Justificativa e Objetivo: A colonização da pele e mucosa por bacilos gran-negativos multirresistentes (MR BGN) em pacientes hospitalizados tem recebido crescente atenção. **Métodos:** Avaliamos a colonização por RM do BGN para determinar o perfil de suscetibilidade antimicrobiana do MR e os genes resistentes em uma população pediátrica. Foi realizado um estudo caso-controle, foram avaliados pacientes considerados colonizados por RM BGN e pacientes não colonizados por RM BGN. As variáveis comparadas entre os grupos foram infecção, cateter venoso central, ventilação mecânica pulmonar, cateter urinário a longo prazo e evolução. A sensibilidade da bactéria aos agentes antimicrobianos foi analisada pela técnica de difusão em disco e a diversidade genética dos isolados pela reação em cadeia da polimerase de sequência intergênica repetitiva

enterobacteriana (ERIC-PCR). **Resultados:** Os pacientes infectados tiveram 4 vezes mais chances de serem colonizados pelo BGN MR do que os pacientes não infectados. Entre as crianças colonizadas pelo MR BGN, a presença de um procedimento invasivo aumentou em 15 vezes as chances de infecção. Em crianças que não foram colonizadas pelo MR BGN, a presença de um procedimento invasivo aumentou em 4 vezes as chances de infecção. Entre as 20 linhagens de BGN MR analisadas, foi encontrada menor resistência ao carbapenem e amicacina. O mecanismo de resistência mais comum foi a produção de enzimas CTXM1, seguidas por CTXM15 e SHV. **Conclusões:** a colonização pela RM do BGN esteve associada à infecção em pacientes pediátricos; portanto, devem ser tomadas medidas para evitar a colonização por MR BGN. Portanto, conhecer o status da colonização bacteriana permanece importante nas enfermarias pediátricas.

Palavras chave: *Enterobacteriaceae; Resistência microbiana a medicamentos; Infecção Hospitalar; Controle de infecção.*

RESUMEN

Justificación y Objetivo: La colonización de la piel y la mucosa por bacilos gran-negativos resistentes a múltiples fármacos (BGN MR) en pacientes hospitalizados ha recibido una atención creciente. **Métodos:** Evaluamos la colonización por BGN MR para determinar el perfil de susceptibilidad antimicrobiana BGN MR y los genes resistentes en una población pediátrica. Se realizó un estudio de casos y controles, se evaluaron pacientes considerados colonizados por BGN MR y pacientes no colonizados por BGN MR. Las variables que se compararon entre los grupos fueron infección, catéter venoso central, ventilación mecánica pulmonar, catéter urinario a largo plazo y desenlace. La sensibilidad de la bacteria a los agentes antimicrobianos se analizó mediante la técnica de difusión en disco, y la diversidad genética de los aislamientos se analizó mediante la reacción en cadena de secuencia polimerasa de secuencia intergénica repetitiva enterobacteriana (ERIC-PCR). **Resultados:** los pacientes con infección tenían 4 veces más probabilidades de ser colonizados por BGN MR que los pacientes no infectados. Entre los niños colonizados por BGN MR, la presencia de un procedimiento invasivo aumentó las probabilidades de infección en 15 veces. En niños que no fueron colonizados por BGN MR, la presencia de un procedimiento invasivo aumentó las probabilidades de infección en 4 veces. Entre las 20 cepas de BGN MR analizadas, se encontró menor resistencia para carbapenem y amikacina. El mecanismo de resistencia más común fue la producción de enzimas CTXM1, seguido de CTXM15 y SHV. **Conclusiones:** la colonización por BGN MR se asoció con infección en pacientes pediátricos; por lo tanto, se deben tomar medidas para prevenir la colonización por BGN MR. Por consiguiente, conocer el estado de la colonización bacteriana sigue siendo importante en las salas de pediatría.

Palabras clave: *Enterobacteriaceae; Farmacorresistencia Microbiana; Infección Hospitalaria; Control de Infecciones.*

INTRODUCTION

Colonization may be the first step of health care-related infection (HCRI), which is acquired soon after admission and manifests during hospitalization or after discharge, when it can be related to hospitalization or hospital procedures.[1,2]

Several factors contribute to the occurrence of HCRI, including the use of invasive device, as central vascular catheters (CVCs) and consequently a greater potential for morbidity and mortality.[3]

The risk factors for gram-negative bacilli (GNB) infection are as follows: use of antibiotics in the previous six months, previous hospitalization (last three months), prior surgery (last 12 months), presence of chronic disease, presence of immunosuppression, and invasive device use (invasive ventilation, central catheters, and urinary catheter).[4,5]

Childhood represents a particularly vulnerable stage of life, with the highest susceptibility to infections, especially in the age group below 2 years, since the immune system is immature. In the hospital environment, the child is exposed to a wide variety of microorganisms that can trigger colonization and infection.[4]

In the United States, 1,003 hospitals reported 20,390 HCRIs with the presence of 22,323 microorganisms in paediatric units between 2011 to 2014. Among all HCRIs, the following pathogens were responsible for more than 60% of those reported: *Staphylococcus aureus* (17%), coagulase-negative staphylococci (17%), *Escherichia coli* (11%), *Klebsiella pneumoniae* and/or *K. oxytoca* (9%), and *Enterococcus faecalis* (8%).[6]

Although the development of antimicrobial resistance is a naturally occurring phenomenon, there is greater selective pressure and dissemination of resistance due to the misuse of antimicrobials and inadequate programmes for infection prevention and control, which favour the transmission of resistance among microorganisms.

Multidrug resistant GNB (MR GNB) are unanimously recognized as one of the most troubling challenges in the field of health care. Their clinical impact is even more worrying in neonatal and paediatric care, where treatment options are limited.[7,8]

For the prevention and control of HCRI, it is necessary to establish MR control policies in the institution and standardization of the implementation and maintenance of invasive devices. The objective of this study was to analyse the association between colonization and HCRI by MR GNB of hospitalized children in the paediatric ward of a university hospital (UH) in southern Brazil.

METHODS

Study population

This is a prospective cohort study of children during hospitalization in a UH. A patient was considered to be colonized by MR GNB when his or her first swab was negative and he or she subsequently presented at least one swab or sterile material that was positive for MR GNB during the same hospital stay. Patients with two or more negative swabs and sterile material without MR GNB isolation at the same hospital stay were considered to be non-colonized by MR GNB.

After the definition of cases (patients colonized by MR GNB) and controls (patients not colonized by MR GNB), a case-control study was performed to analyse the variables: infection, central venous catheter (CVC), lung mechanical ventilation (MV), long-term urinary catheter (LTUC), and outcome.

The study was approved by the local board of ethics, nº 15415413.4.0000.5231 of April 22, 2016. All mothers of recruited patients who were hospitalized in the Paediatric Units of the University Hospital of Londrina were asked to carefully read and sign an informed consent and the present research was performed in accordance with the ethical standards of the 1964 Declaration of Helsinki and its later amendments. All samples were coded to protect patient anonymity.

Inclusion and exclusion criteria

Among 687 patients hospitalized from January to December 2016 at the Paediatric Ward and Paediatric ICU

of the UH, all children between 0 and 12 years old who were colonized by MR GNB were included in this study. No cultures were collected for 432 children. Cultures were collected from 255 children, of whom 106 were characterized as the control group. A total of 149 children had positive cultures, of whom 114 were excluded because it was not possible to define the moment of colonization and 35 children were characterized as the case group.

MR GNB included *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* resistant to carbapenem and Enterobacteria resistant to 3rd or 4th generation cephalosporins or monobactams.[9]

The infection data were obtained from Hospital Infection records of the Hospital Infection Control Commission.

Microbiological procedures

Sample collection

Two swabs were collected using the aseptic technique – one swab for nasal, oral, axillary and inguinal regions, and another swab for inguinal and rectal regions. The swabs were transported to the microbiology laboratory in Stuart medium, within a maximum of 4 hours.

Phenotypic isolation and identification of bacteria

The biological samples contained in the swabs were inoculated in selective media for each target bacterium of the study. Concurrently, the species were identified using an automated method with the Vitek® platform (BioMérieux). Bacteria were stored in an appropriate culture medium containing 20% glycerol at -80°C.

Determination of the profile of sensitivity to antimicrobial agents

The sensitivity of the bacteria to the antimicrobial agents was analysed using the disc-diffusion technique according to the recommendations of the Clinical and Laboratory Standard Institute[10] using the recommended antimicrobial discs. The following disks were used: ciprofloxacin (5 mcg), gentamicin (10 mcg), amikacin (30 mcg), sulfamethoxazole + trimethoprim (25 mcg), cephalothin (30 mcg), amoxicillin (30 mcg), piperacillin + tazobactam (110 mcg), cefoxitin (30 mcg), aztreonam (30 mcg), cefotaxime (30 mcg), ceftazidime (30 mcg), cefepime (30 mcg), ertapenem (10 mcg), imipenem (10 mcg), and meropenem (10 mcg).

ESBL detection

The presence of Extended Spectrum Beta-Lactamase (ESBL) was confirmed by the double-disc approximation and combined disc test using Oxoid® discs (Basingstoke, Hampshire, England). *Klebsiella pneumoniae* ATCC 700603 and *Escherichia coli* 25922 were used as positive and negative controls, respectively, according to Clinical Laboratory Standards Institute (CLSI).[10]

Molecular analysis

DNA extraction

The DNA was prepared for single polymerase chain reaction (PCR) using the boiling method. Three to five bacterial colonies isolated on tryptic soy broth (TSB) were suspended in 0.5 ml of ultra-pure water and boiled for 10 min, followed by heat shock for 5 min. The suspension was centrifuged at 10,000 rpm for 10 min, and the supernatant was used.

Detection of β-lactamases

Samples that presented positive phenotypic test were characterized genotypically by PCR. Multiplex PCR was conducted using the TopTaq® Master Mix Kit (QIAGEN®) with the following amplification conditions: initial 5-min denaturation at 94ºC; 30 cycles of 25 sec at 94°C denaturation, 40 sec at 52°C for annealing, and 50 sec at 72°C extension; and a final extension of 6 min at 72°C for the $bla_{\text{CTX-M1}}$, $bla_{\text{CTX-M2}}$, $bla_{\text{CTX-M8}}$, $bla_{\text{CTX-M9}}$, and $bla_{\text{CTX-M25 genes.}}$[11] PCR for the $bla_{\textit{CTX-M15 gene}}$ was performed according to Leflon-guibout *et al.*[12] The reaction was subjected to 30 cycles of 94ºC for 7 min, 94ºC for 1 min, 50°C for 1 min, and 72°C for 1 min and a final extension at 72°C for 6 min. PCR for the bla_{KPC} gene was performed according to Bradford *et al.*[13] The reaction was subjected to 94°C for 5 min, followed by 30 cycles at 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min and a final extension at 72°C for 7 min. PCR for the bla_{TEM} and bla_{SHV} genes was performed according to Arlet and Philippon.[14]

Agarose gel electrophoresis

The products of the single PCR were analysed on a 1.0% agarose gel in 1x Tris/Borate/EDTA (TBE) solution. For the agarose gel application, 4 µL of buffer sample was added, and electrophoresis was performed at 80 volts for 40 min. The 100-bp molecular weight marker (Invitrogen®) was applied to the gel to determine the size of the obtained fragments. The gel was then incubated with ethidium bromide solution (0.08 µL/100 mL) for 15 min and visualized under ultraviolet light.

Polymerase chain reaction based on intergenic repetitive sequences in enterobacteria (ERIC –PCR)

The PCR was performed with primers ERIC1R (3'CACTTAGGGGTCCTCGAATGTA-5') and ERIC2 (5'-AAGTAAGTGACTGGGGTGAGCG-3') at a concentration of 50 pmol, as described by Versalovic et al.[15] The amplification product was subjected to 1.5% agarose gel electrophoresis with TBE buffer (tris base, boric acid and EDTA). The device was set to 100 V and 400 mA. The analysis was performed by gel staining with ethidium bromide (0.5 µg/mL) and visualization under a transilluminator with ultraviolet light followed by imaging for documentation.

Statistical analysis

The data were entered into the Microsoft Office Excell 2007 database, and statistical analysis was performed using the Statistical Package for Social Sciences (SPSS - IBM Corp., New York, USA), version 20 for Windows. The means, medians and standard deviations of continuous variables were calculated. Categorical variables were analysed by the chi-square test or Fisher's exact test where appropriate, and continuous variables were analysed by the Mann-Whitney test. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Among the 687 inpatients hospitalized from January to December 2016 at the Paediatric Ward and Paediatric UTI of the UH, all children aged between 0 and 12 years who were colonized by MR GNB were included in this study. No cultures were collected for 432 children. Cultures were collected from 255 children, of whom 106 were characterized as the control group. A total of 149 children had positive cultures, of whom 114 were excluded because it was not possible to define the moment of colonization and 35 were characterized as the case group. In the 35 patients in the case group, 40 MR GNB were isolated: 10 samples with *Klebsiella sp* (25%), 11 with *Escherichia coli* (27.5%), 5 with *Enterobacter* spp (12.5%), 4 with *Serratia* spp (10.0%), 1 with *Proteus* spp (2.5%), and 1 with *Citrobacter* spp (2.5%). In 8 of these patients, 3 *Enterobacter* spp resistant to carbapenems (CR) (7.5%), 2 *Acinetobacter* spp CR (5.0%), 2 *Klebsiella* spp CR (5.0%), and 1 *Serratia* spp CR (2.5%) were isolated (Fig. 1).

Table 1. Analysis of the association of colonization by multidrug resistant gram- negative bacilli (MR GNB) with clinical-demographic variables, University Hospital of Londrina/PR- Brazil, January to December 2016.

	Patients colonized by MR GNB (n=35)	Patients not colonized by MR GNB (n=106)	Odds Ratio (IC 95%)	*p*
Average age (months)	24.8 (0.2-82.6)	18.5 (1.4-63.6)	-	0.916
Sex				
Female	16 (45.7%)	42 (39.6%)	1.283 (0.594-2.773)	0.525
Male	19 (54.3%)	64 (60.4%)		
Length of hospital stay (days)	17.0 (10.0-48.0)	13.5 (8.0-25.5)	-	0.088
Presence of infection				
Yes	14 (40.0%)	15 (14.2%)	4.044 (1.696-9.647)	0.001
No	21 (60.0%)	91 (85.8%)		
Exposure to invasive procedure				
Yes	7 (20.0%)	22 (20.8%)	0.955 (0.368-2.473)	0.924
No	28 (80.0%)	84 (79.2%)		
Type of procedure				
None	28 (80.0%)	84 (79.2%)	-	0.954
MV	04 (11.4%)	12 (11.3%)		
CVC	0 (0.0%)	1 (1.0%)		
LTUC	03 (8.6%)	09 (8.5%)		
Outcome				
Discharge	35 (100.0%)	103 (97.2%)	1.029 (0.996-1.063)	0.574
Death	00 (0.0%)	3 (2.8%)		

Note. MV mechanical pulmonary ventilation, CVC central vascular catheter, LTUC long-term urinary catheter. The categorical data were evaluated by Chi-square or Fisher's Exact.

The demographic and clinical data for the patients who were colonized and not colonized by MR GNB are presented in table 1. The median age of the patients was 19.8 months (interquartile range [IQR; 25 and 75] 1 - 66 months). The median time of hospitalization was 15 days (IQR [25 and 75] 8 - 31 days). The time to the swab becoming positive was 3 to 51 days, with a median of 9 days (IQR [25 and 75] 6 - 25 days).

Patients with infection were 4 times more likely to present MR GNB colonization when compared to non-infected patients (OR: 4.044; CI 1.696 - 9.647). No differences were found between the groups that were colonized or not colonized by MR GNB in relation to the presence of the procedure (p=0.924), type of procedure (p=0.954), and outcome (p=0.574) (Table 1).

Demographic and clinical data for the patients colonized by MR GNB according to the presence of infection are presented in table 2. Male subjects were 16% less likely than females subjects to have infection (OR: 0.16, CI 0,36-0.72).

Patients who were colonized and who had undergone an invasive procedure were 15 times more likely to have infection than patients without an invasive procedure (OR: 15.0; CI 1.5-145.2).

Most patients without infection had no invasive procedure, while 42.9% of the patients with infection used MV or LTUC.

All patients colonized by MR GNB were discharged regardless of the presence or absence of infection.

Data from patients who were not colonized by MR GNB according to the presence or absence of infection are presented in table 3. Non-colonized patients who underwent some type of procedure were 4 times more likely to present infection than patients without any procedure. Regarding the type of procedure, 46.7% of the non-colonized and infected patients

Table 2. Analysis of the association of infection with the demographic and clinical data of patients colonized by multiresistant gram-negative bacilli, University Hospital of Londrina/PR- Brazil, January to December 2016.

	Presence of infection (n=14)	Absence of infection (n=21)	Odds Ratio (IC 95%)	*p*
Sex	10 (71.4%)	06 (28.6%)	0.16 (0.360-0.715)	0.018
Female	04 (28.6%)	15 (71.4%)		
Male				
Exposure to invasive procedure	6 (42.9%)	1 (4.8%)	15.0 (1.500-145.200)	0.010
Yes	8 (57.1%)	20 (95.2%)		
No				
Type of procedure	08 (57.1%)	20 (95.2%)		
None	2 (14.3%)	1 (4.8%)	-	0.015
MV	0 (0.0%)	0 (0.0%)		
CVC	4 (28.6%)	0 (0.0%)		
LTUC				
Outcome	14 (100.0%)	21 (100.0%)	-	-
Discharge	0 (0.0%)	0 (0.0%)		
Death				

Note. MV mechanical pulmonary ventilation, CVC central vascular catheter, LTUC long-term urinary catheter. The categorical data were evaluated by Chi-square or Fisher's Exact and presented.

Table 3. Analysis of the association of infection with the demographic and clinical data of patients not colonized by multiresistant gram-negative bacilli, University Hospital of Londrina/PR- Brazil, January to December 2016.

	Presence of infection (n=15)	Absence of infection (n=91)	Odds Ratio (IC 95%)	*p*
Sex				
Female	7 (46.7%)	35 (38.5%)	0.714 (0.238-2.140)	0.547
Male	8 (53.3%)	56 (61.5%)		
Exposure to invasive procedure				
Yes	7 (46.7%)	15 (16.5%)	4.43 (1.396-14.089)	0.008
No	8 (53.3%)	76 (83.5%)		
Type of procedure				
None	8 (53.3%)	76 (83.5%)	-	0.012
MV	3 (20.0%)	10 (11.0%)		
CVC	0 (0.0%)	1 (1.1%)		
LTUC	4 (26.7%)	4 (4.4%)		
Outcome				
Discharge	14 (93.3%)	89 (97.8%)	0.32 (0.027-3.704)	0.370
Death	1 (6.7%)	2 (2.2%)		

Note. MV mechanical pulmonary ventilation, CVC central vascular catheter, LTUC long-term urinary catheter. The categorical data were evaluated by Chi-square or Fisher's Exact.

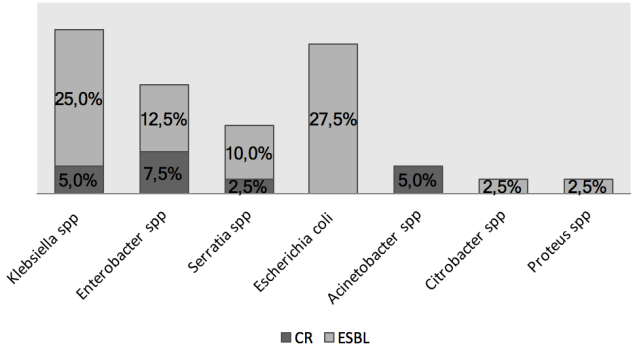

Figure 1. Presentation of multidrug resistant gram-negative bacilli (n = 35), University Hospital of Londrina/PR- Brazil, January to December 2016.

underwent MV or LTUC, while 16.5% of the non-infected patients underwent MV, CVC, or LTUC. Only one patient colonized by *Klebsiella* spp had this microorganism isolated in urine, and the patient was considered to have a urinary infection.

Of the 35 patients who were colonized with MR GNB, the molecular study and sensitivity profile of ESBL-producing enterobacteria were performed in 20 representative samples. Among these samples, 4 (20.0%) had *E. cloacae*, 6 (30.0%) *K. pneumoniae*, 9 (45.0%) *E. coli*, and 1 (5.0%) had *Citrobacter* spp (Fig. 1).

Among the strains analysed, we observed 65% resistance to ciprofloxacin, 40% resistance to sulfamethoxazole trimethoprim and piperacillin-tazobactam, 25% resistance to amikacin, 15% to ertapenem, and 0% resistance to imipenem and/or meropenem.

The genotypic analysis of MR GNB is presented in Figure 2.

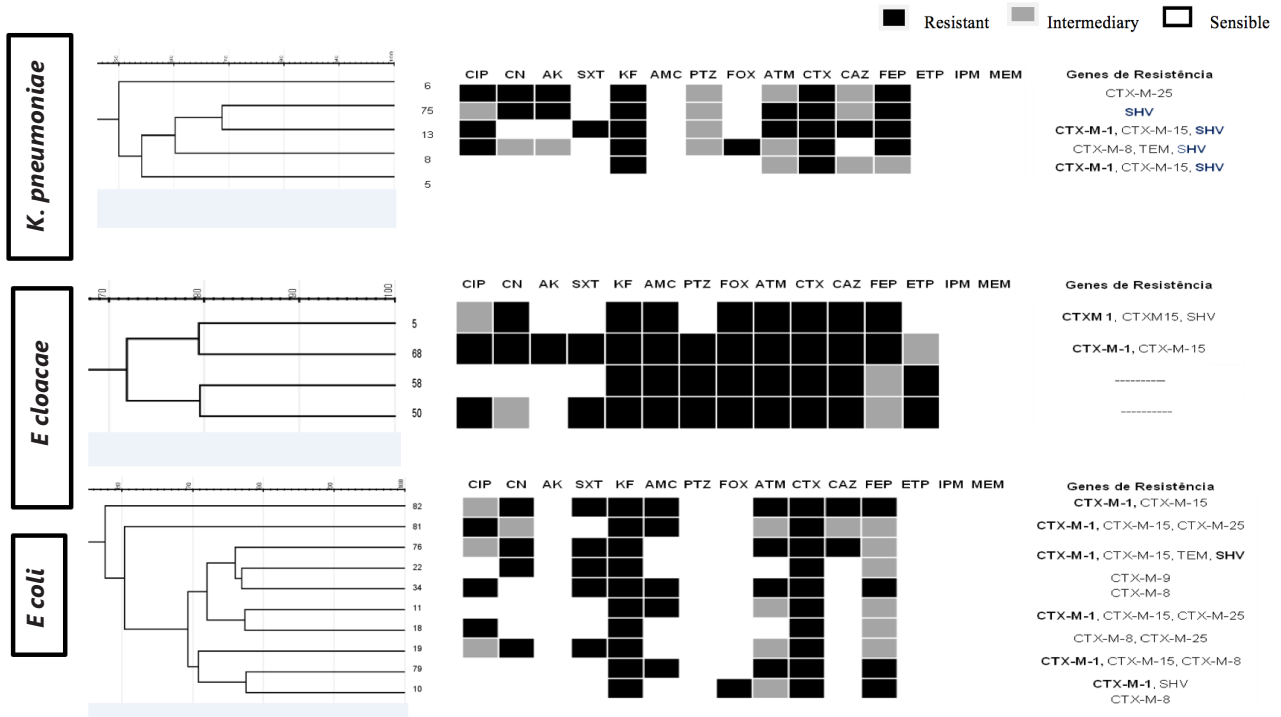

Figure 2. Dendrogram, antimicrobial resistance profile and main resistance enzymes of multidrug resistant gram-negative bacilli
CIP: ciprofloxacin, CN: gentamicin, AK: amikacin, SXT: trimethoprim-sulfamethoxazole, KF: cephalotin, AMC: amoxicillin, PTZ: piperacillin-tazobactam, FOX: cefoxitin, ATM: aztreonam, CTX: cefotaxime, CAZ: ceftazidime, FEP: cefepime, ETP: ertapenem, IPM: imipenem, MEM: meropenem

In 7 samples (35%), the microorganisms presented 3 different resistance enzymes. The CTXM1 enzyme was present in 50% of the samples. Among the 6 samples of *Klebsiela* spp, the SHV enzyme was detected in 5 (83.3%) samples, CTXM1 in 3 (50%) samples, and TEM in 1 (16.7%) sample. In four samples of *Klebsiella* spp, an association of two or more enzymes was detected.

In two samples with ESBL *E. cloacae* isolate, it was not possible to define the enzyme responsible for the combined resistance.

DISCUSSION

In our study, we observed a high frequency of MR GNB, and the patients presented a median age and frequency of colonization that were higher than those described in the literature. A survey performed with 111 African paediatric patients with a median age of 8 months (IQR 25 and 75: 4 and 14 months) undergoing cardiac surgery identified colonization by ESBL MR GNB in 17 (15%) patients, while 94 (85%) patients were not colonized. However, as in our study, the isolated species were *K. pneumoniae* in 9 (53%) patients, *E. coli* in 6 (35%) patients, and *E. cloacae* in 2 (12%) patients.[16] Lautenbach *et al.* conducted a study in North American adults and found no differences between the colonized and non-colonized groups with respect to age and sex. They also showed that colonized patients were more likely than non-colonized patients to have infection, longer hospitalizations prior to infection, and a CVC or urinary catheter.[17] As weaknesses of the present study, we highlight the small number of patients with CVC.

Research on colonization by ESBL-producing microorganisms in Polish children subjected to cardiac surgery showed colonization in 16% of them.[18] French patients in an intensive care unit (ICU) had 25% colonization by ESBL GNB, and hospitalized Korean patients had 28.2% colonization by ESBL GNB.[19,20]

In the present study, patients who were colonized by MR GNB had higher odds of infection; similar results were found by other researchers. Cheikh *et al.* showed that among the patients who were colonized by ESBL, 4 (23.5%) developed postoperative infection; however, only 1% of non-colonized patients developed postoperative infection.[16] The microorganisms that were responsible for the infections in the colonized patients were *K. pneumoniae* (2 patients; 50%) and *E. coli* (2 patients; 50%). The chance of developing infection in colonized

patients was 22 times higher than in non-colonized patients (CI 95% 8.37-58.5), and the only non-colonized patient who developed infection demonstrated infection by ESBL *E. cloacae*.

Another study showed that patients colonized by *E. coli* and *K. pneumoniae* were more likely than the controls to have infection, longer hospitalizations prior to infection, and use of a CVC or urinary catheter.[17]

Ben-Ami *et al.* showed that colonized individuals had a higher risk of bacteraemia than non-colonized individuals.[21] Cheikh *et al.* also showed an association between previous colonization with ESBL and the occurrence of infection after surgery in paediatric patients.[16] These results are in agreement with those found in our study, in which patients colonized with MR GNB were 4 times more likely than non-colonized patients to have infection.

Bacterial resistance has been a challenge in clinical practice. Among the treatment options for ESBL MR GNB infections, piperacillin-tazobactam has often been associated with therapeutic failure, necessitating a therapeutic change for carbapenems. In turn, the indiscriminate use of carbapenems has led to their resistance and the need to prescribe new drugs, such as tigecycline, and older drugs, such as colistin.[22, 23]

In the present study, the enzyme CTXM was detected in half of the samples with the GNB isolate. The presence of CTXM *E. coli* clones has been associated with severe invasive infections.[24] The most widely found extended spectrum beta-lactamases are CTXM, SHV, and TEM, with increasing KPCs.[23,25]

According to Pereira *et al*., among 2563 cases of bloodstream infections from June 2007 to March 2010, GNB was isolated from 49% of the samples, and *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa* were the main isolated microorganisms.[26] The resistance of piperacillin-tazobactam ranged from 25-55.6%, and resistance to carbapenems ranged from 2-23%; in our study, 20% of the total samples were resistant to imipenem and/or meropenem (CR). They reported that among the isolates of *K. pneumoniae,* the presence of CTXM, and SHV and TEM was 63.6%, 45.4% and 28%, respectively, whereas in our study, 6 samples of *Klebsiella pneumoniae* were isolated, and SHV was detected in most of these samples, followed by CTXM1.

In conclusion, MR GNB colonization was associated with infection in paediatric patients, and the presence of invasive procedures (MV and LTUC) was a facilitator for infection in children colonized by MR GNB. Therefore, measures should be taken to prevent MR colonization in hospitalized children.

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REFERENCES

1. *McKibben L, Horan T, Tokars JI, et al. Guidance on public reporting of healthcare-associated infections: Recommendations of the healthcare infection control practices advisory committee. Am J Infect Control 2005; 33:217-26. doi: 10.1016/j.ajic.2005.04.001*
2. *Padoveze M C, Fortaleza CMCB. Healthcare-associated infections: Challenges to public health in Brazil. Rev Saude Publica 2014; 48:995-1001. doi: 10.1590/S0034-8910.2014048004825*
3. *Rosado V, Camargos PAM, Anchieta LM, et al. Risk factors for central venous catheter-related infections in a neonatal population systematic review. J Pediatr 2018; 18:3-14. doi: 10.1016/j.jped.2017.03.012*
4. *Paes ARM, Câmara JT, Santos DAS, et al. Epidemiological study of cross infection in Intensive Care. REUFPI 2014; 3:0-7. doi: 10.26694/reufpi.v3i4.1858*
5. *Vardakas KZ, Rafailidis PI, Konstantelias AA, et al. Predictors of mortality in patients with infections due to multi-drug resistant gram negative bacteria: The study, the patient, the bug or the drug? J Infect 2013; 66:401-414. c. doi: 10.1016/j.jinf.2012.10.028*
6. *Lake JG, Weiner LM, Milstone AM, et al. Pathogen Distribution and Antimicrobial Resistance Among Pediatric Healthcare-Associated Infections Reported to the National Healthcare Safety Network, 2011–2014. Infect Control Hosp Epidemiol 2018; 39:1. doi: 10.1017/ice.2017.236*
7. *Gray JW, Ubhi H, Milner P. Antimicrobial treatment of serious gram-negative infections in newborns. Curr Infect Dis Rep 2014;16:400–408. doi: 10.1007/s11908-014-0400-6.*
8. *Giuffre M, Geraci D, Bonura C, et al. The increasing challenge of multidrug-resistant gram-negative bacilli: results of a 5-year active surveillance program in a neonatal intensive care unit. Medicine 2016; 95:1–10. doi: 10.1097/MD.0000000000003016*

9. *Sievert D, Ricks P, Edwards J, et al. Antimicrobial-resistant pathogens associated with heathcare-associated infections: summary of data reported to the National Heathcare safety Network at the Centers for Disease Control and Prevention.2009-2010. Infect. Control Hosp Epidemiol 2013;34(1):1-14. doi: 10.1086/668770*
10. *Clinical Laboratory Standards Institute. Performance standards for Antimicrobial susceptibility testing: Seventh Informational Supplement M 100-S19. CLSI: Wayne, Pennsylvania USA. 2016.*
11. *Woodford N, Fagan EJ, Ellington MJ. Multiplex PCR for rapid detection of genes encoding CTX-M extended-spectrum β-lactamases. J Antimicrob Chemother 2006;57:154-155. doi: 10.1093/jac/dki412*
12. *Leflon-Guibout V, Jurand C, Bonacorsi S, et al. Emergence and spread, of three clonally related virulent isolates of CTX -M-15-producing Escherichia coli with variable resistance to aminoglycosides and tetracycline in a French geriatric hospital. Antimicrob Agents Chemother 2004;48(10):3736–3742. doi: 10.1128/AAC.48.10.3736-3742.2004*
13. *Bradford PA, Bratu S, Urban C, et al. Emergence of Carbapenem-Resistant Klebsiella Species Possessing the Class A Carbapenem-Hydrolyzing KPC-2 and Inhibitor-Resistant TEM-30-β Lactamases in New York City. Clin Infect Dis 2004; 39(1):55–60. doi: 10.1086/421495*
14. *Arlet G, Philippon A. Construction of polymerase chain reaction and use of intragenic DNA probes for three main types of transferable Beta-lactamases (TEM,SHV,CARB). FEMS Microbiol 1991; 66:19-25. doi: 10.1016/0378-1097(91)90414-6*
15. *Versalovic J, Koeuth T, Lupski JR. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. Nucleic Acids Res 1991; 19(24):6823-31. doi: 10.1093/nar/19.24.6823*
16. *Cheikh A, Belefquih B, Chajai Y, et al. Enterobacteriaceae producing extended-spectrum β-lactamases (ESBLs) colonization as a risk factor for developing ESBL infections in pediatric cardiac surgery patients: "retrospective cohort study". BMC Infect Dis 2017; 17(1),1–6. doi: 10.1186/s12879-017-2346-4*
17. *Lautenbach E, Patel JB, Bilker WB, et al. Extended-spectrum ß-lactamase producing Escherichia coli and Klebsiella pneumoniae: risk factors for infection and impact of resistance on outcomes. Clin Infect Dis 2001; 32(8),1162-1170. doi: 10.1086/319757*
18. *Jaworski R, Haponiuk I, Steffens M, et al. Colonization of multidrug resistant pathogens in a hybrid pediatric cardiac surgery center. Arch Med Sci 2016; 12(3):639–44. doi: 10.5114/aoms.2016.59937*
19. *Razazi K, Derde LPG, Verachten M, et al. Clinical impact and risk factors for colonization with extended-spectrum β-lactamase-producing bacteria in the intensive care unit. Intensive Care Med 2012; 38:1769–78. doi: 10.1007/s00134-012-2675-0*
20. *KO YJ, Moon HW, Hur M, et al. Fecal carriage of extended-spectrum beta-lactamase-producing Enterobacteriaceae in Korean community and hospital settings. Infection 2013; 41,9–13. doi: 10.1007/s15010-012-0272-3*
21. *Ben-Ami R., Schwaber MJ, Navon-Venezia S, et al. Influx of extended-spectrum beta-lactamase-producing enterobacteriaceae into the hospital. Clin Infect Dis 2006; 42:925–934. doi: 10.1086/500936*
22. *Boucher HW, Talbot GH, Bradley JS, et al. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis 2009;48(1):1-12. doi: 10.1086/595011*
23. *Hsu AJ, Tamma PD. Treatment of multidrug-resistant gram-negative infections in children. Clin Infect Dis 2014; 58(10):1439-1448. doi: 10.1093/cid/ciu069*
24. *Pitout JD, Laupland KB, Church D, et al. Virulence Factors of Escherichia coli Isolates That Produce CTX-M-type extended-spectrum beta-lactamases. Antimicrob Agents Chemother 2005; 49(11):4667–4670. doi: 10.1128/AAC.49.11.4667-4670.2005*
25. *Lukac PJ, Bonomo RA, Logan LK. Extended-spectrum b-lactamase-producing Enterobacteriaceae in children: old foe, emerging threat. Clin Infect Dis 2015; 60(9):1389-1397. doi: 10.1093/cid/civ020*
26. *Pereira CA, Marra AR, Camargo LF, et al. Nosocomial Bloodstream Infections in Brazilian Pediatric Patients: Microbiology, Epidemiology, and Clinical Features. PLoS ONE 2013; 8(7):8-13. doi: 10.1371/journal.pone.0068144*