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Original Article

Antimicrobial susceptibility of ESKAPE pathogens in the SS. Antonio e Biagio e Cesare Arrigo Hospital's catchment area: December 2021 - July 2022

Antibiotico-resistenza dei patogeni del gruppo ESKAPE isolati nel bacino di utenza dell'Ospedale SS. Antonio e Biagio e Cesare Arrigo di Alessandria nel periodo dicembre 2021 - luglio 2022

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Key words: minimum inhibitory concentration, antibiogram, antibiotic resistance, epidemiology.

ABSTRACT

Background and Aims: this report describes the minimum inhibitory concentration distributions of the main drugs used against ESKAPE pathogens infection, recovered from patients treated between December 2021 and July 2022 at SS. Antonio e Biagio e Cesare Arrigo Hospital.
Materials and Methods: data were extracted by a new Laboratory Information System implemented in mid-November 2021.
Results: after exclusion of colonization: i) 56% of *Enterococcus faecium* was susceptible to teicoplanin and vancomycin; ii) 74% of *Staphylococcus aureus* was susceptible to methicillin; iii) 55.3% of *Klebsiella pneumoniae* was susceptible to cefotaxime, ceftazidime and cefepime and 93.2% of KPC was susceptible to ceftazidime/avibactam; iv) no *Acinetobacter baumannii* strains were resistant to colistin; v) 88.9% of *Pseudomonas aeruginosa* was susceptible to ceftolozane/tazobactam and 92.2% to ceftazidime/avibactam; vi) among *Enterobacter* species, 84.6% was susceptible to cefepime and 87.1% to ciprofloxacin.
Conclusions: periodic reporting of local antibiotic resistance is an adjunctive tool to help the choice of antimicrobial therapy.

Introduzione: questo report descrive le distribuzioni delle concentrazioni minime inibenti dei principali antibiotici utilizzati in corso di infezione sostenuta da patogeni del gruppo ESKAPE, isolati da pazienti ricoverati nel periodo Dicembre 2021 - Luglio 2022 presso l'Ospedale SS. Antonio e Biagio e Cesare Arrigo di Alessandria.
Materiali e Metodi: i dati sono stati estratti mediante un nuovo sistema informativo di laboratorio implementato a metà Novembre 2021.
Risultati: dopo l'esclusione delle colonizzazioni, abbiamo documentato le seguenti percentuali di sensibilità: il 56% degli isolati di *Enterococcus faecium* a teicoplanina e vancomicina; il 74% degli isolati di *Staphylococcus aureus* a meticillina; il 55,3% degli isolati di *Klebsiella pneumoniae* a cefotaxime, ceftazidime e cefepime ed il 93,2% degli isolati di *K. pneumoniae* produttori di *Klebsiella pneumoniae* carbapenemases (KPC) a ceftazidime/avibactam. Non sono stati isolati ceppi di *Acinetobacter baumannii* resistenti a colistina; l'88,9% degli isolati di *Pseudomonas aeruginosa* è risultato sensibile a ceftolozane/tazobactam ed il 92,2% a ceftazidime/avibactam. Nell'ambito del genere Enterobacter, l'84,6% degli isolati è risultato sensibile a cefepime e l'87,1% a ciprofloxacina.
Conclusioni: il continuo monitoraggio della prevalenza locale di resistenza agli antibiotici è un utile strumento di aiuto nella scelta di una corretta terapia antimicrobica.

INTRODUCTION

Antibiotic resistance is a great problem in worldwide hospitals[1] that impacts on mortality and health care costs.[2] The causes underlying antibiotic resistance have been mostly identified in extensive agricultural use and inappropriate prescribing.[3] The most frequently isolated and most important bacteria regarding virulence and antibiotic resistance in the hospital setting are *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species, also called ESKAPE pathogens.[4] The antimicrobial stewardship and the periodic reporting of antimicrobial resistance patterns are consid-

ered two of the most effective strategies to fight back against antimicrobial resistance.[5] The European Antimicrobial Resistance Surveillance Network (EARS-Net) is the largest publicly funded system for antimicrobial resistance surveillance in Europe.[6] Some authors also suggest carrying out a periodic tracking of local antibiotic resistance,[7,8] to check the effectiveness of the strategies in order to contain antimicrobial resistance and to implement more effective and timely changes. The aim of this report is to evaluate the prevalence of antimicrobial resistance of ESKAPE pathogens infection, isolated from patients treated between December 2021 and July 2022 at SS. Antonio e Biagio e Cesare Arrigo Hospital and to provide an adjunctive tool to help selecting antimicrobial therapy.

MATERIALS AND METHODS

Identification and antimicrobial susceptibility testing

This is a cross-sectional study regarding all ESKAPE pathogens isolates obtained from biological samples of inpatients and outpatients between December 2021 and July 2022. Isolates identification was performed by Vitek 2® system (bioMérieux, Marcy l'Etoile, France) or by matrix-assisted laser desorption ionization–time of flight mass spectrometry Vitek® MS (bioMérieux). Antimicrobial susceptibility testing was mainly performed by Vitek 2® system and in case of unusual phenotype, it was confirmed by Minimum Inhibitory Concentration (MIC) gradient strip tests (Etest: bioMérieux) and/or broth microdilution (Micronaut-S: Merlin Diagnostika GmbH, Bornheim, Germany). In case of MIC values suggesting carbapenemase production, the isolates were tested using Xpert Carba-R assay (Cepheid, Sunnyvale, CA, USA). EUCAST version 11.0[9] and 12.0[10] were used to interpret MIC values.

Data extraction

We chose the period December 2021 – July 2022 due to the implementation in mid-November 2021 of a new Laboratory Information System provided with specific requirements for the Microbiology Laboratory (Concerto: Dedalus Healthcare Systems Group SpA, Firenze, Italy), That allows not only the management of the clinical specimens, but also highly configurable epidemiological reports.

Data analysis

Categorical variables were expressed as absolute numbers and percentages. The MIC values were expressed in mg/L on the x-axis and the frequency on the y-axis. Microsoft Excel 2010 (Microsoft Corporation, Redmond, USA; available from: https://office.microsoft.com/excel) was used for quantitative analysis.

RESULTS

Enterococcus faecium

A total of 269 isolates of *E. faecium* are described in Table 1, the MIC distributions for teicoplanin, vancomycin, linezolid and tigecycline are in Figure 1.

After excluding rectal swabs, among the 125 isolates, 55 (44%) had MIC-resistant values >2 mg/L for teicoplanin and >4 mg/L for vancomycin. All isolates were resistant to ampicillin, amoxicillin/clavulanate, ampicillin/sulbactam and imipenem.

Staphylococcus aureus

From the same number of samples, a total of 506 isolates of *S. aureus* are described in Table 2. Figure 2 shows the MIC distributions for penicillin G, ceftaroline, glycopeptides, linezolid, daptomycin, tigecyclin and rifampicin. One hundred and seventy-seven isolates (34.9%) were methicillin-resistant (cefoxitin screening test positive). Not considering nasal swabs, the percentage of cefoxitin-resistant isolates was 26% (115/442).

Klebsiella pneumoniae

A total of 697 isolates of *K. pneumoniae* are described in Table 3. Different Vitek 2® cards were used in relation to the site of infection and so, not all isolates were tested for the same antimicrobials. The MIC distributions for the main beta-lactam antibiotics, amikacin, ciprofloxacin, trimethoprim/sulfamethoxazole and colistin are reported in Figure 3A. Figure 3B shows the mechanisms of resistance identified.

After exclusion of rectal swabs: i) 219/560 (39.1%) were resistant to both amoxicillin/clavulanic acid and piperacillin/tazobactam; ii) 208/568 (36.6%) were resistant to cefotaxime, ceftazidime and cefepime; iii) among these 208, 98/568 (17.3%) were also resistant to both imipenem and meropenem; iv) among the 95 *K. pneumoniae* Carbapenemase (KPC) producers, 44 were tested for ceftazidime/avibactam and 41/44 (93.2%) had MIC values ≤8 mg/L, susceptible; v) among the 553 isolates tested for amikacin, 540 (97.6%) had MIC values ≤8 mg/L, overall classifiable as "isolates without resistance mechanisms".

Table 1. Number of isolates of *Enterococcus faecium* according to sample.

Sample	N
Rectal swab	144
Urine culture	54
Blood culture	29
Wound/ulcer swab	25
Biopsy	17
Total	269

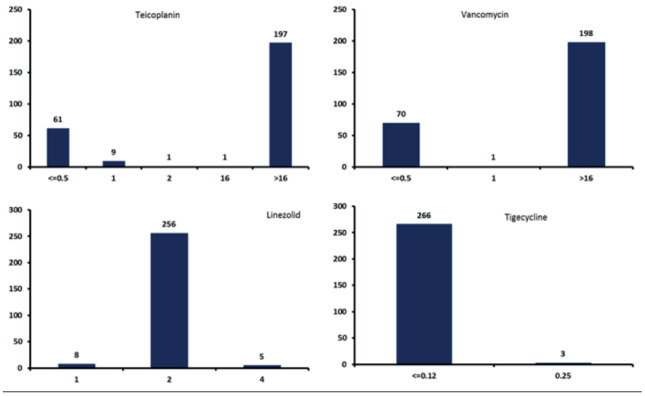

Figure 1. Minimum Inhibitory Concentration (MIC) distributions for glycopeptides, linezolid and tigecycline against *Enterococcus faecium*. MIC values are expressed in mg/L.

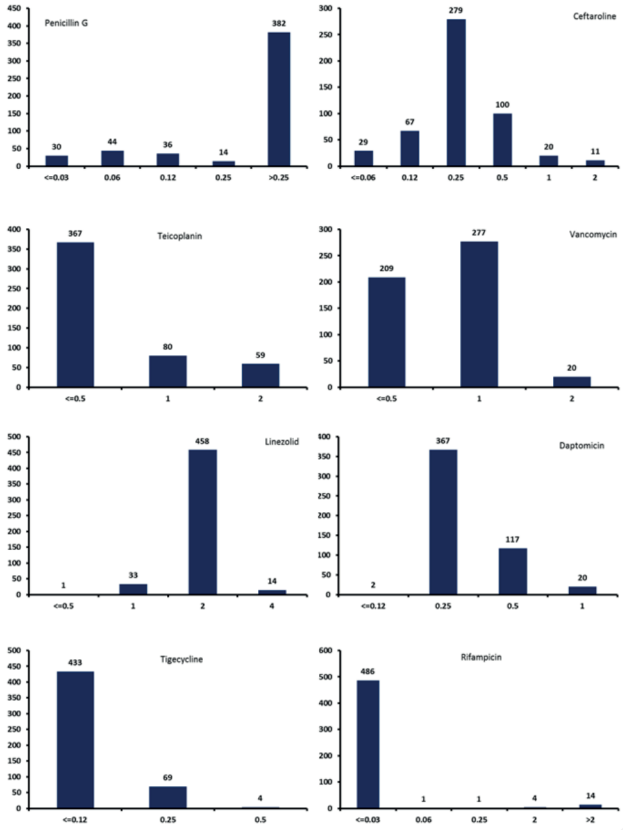

Figure 2. Minimum Inhibitory Concentration (MIC) distributions for penicillin G, ceftaroline, glycopeptides, linezolid, daptomycin, tigecyclin and rifampicin against *Staphylococcus aureus*. MIC values are expressed in mg/L.

Table 2. Number of isolates of *Staphylococcus aureus* according to sample.

Sample	N
Wound/ulcer swab	205
Blood culture	77
Biopsy	67
Nasal swab	64
Bronchoalveolar lavage/Bronchial aspirate	39
Urine culture	39
Central venous catheter tip culture	5
Cerebrospinal fluid culture	5
Conjunctival swab	3
Ear swab	2
Total	506

Table 3. Number of isolates of *Klebsiella pneumoniae* according to sample.

Sample	N
Urine culture	400
Rectal swab	128
Blood culture	64
Wound/ulcer swab	38
Bronchoalveolar lavage/Bronchial aspirate	37
Biopsy	21
Central venous catheter tip culture	9
Total	697

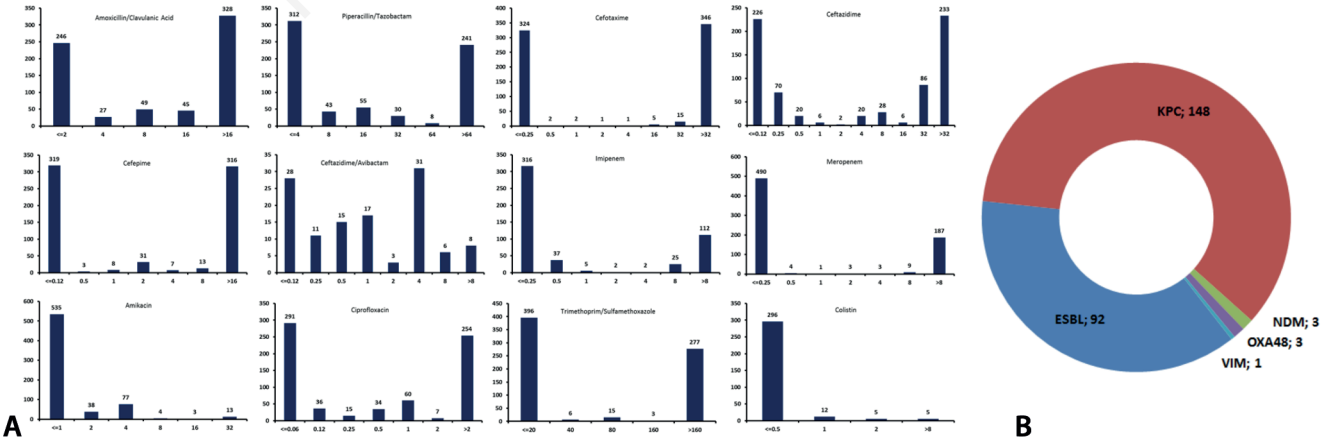

Figure 3. A) Minimum Inhibitory Concentration (MIC) distributions for main beta-lactam antibiotics, amikacin, ciprofloxacin, cotrimoxazole and colistin used against *Klebsiella pneumoniae*. MIC values are expressed in mg/L. B) *Klebsiella pneumoniae* isolates with identified mechanisms of resistance to beta-lactams.

Acinetobacter baumannii

A total of 112 isolates of *A. baumannii* are described in Table 4. As for *K. pneumoniae*, different Vitek 2® cards were used in relation to the site of infection and so, not all isolates were tested for the same antimicrobials. Figure 4 shows the MIC distributions for meropenem, ciprofloxacin, aminoglycosides, colistin and trimethoprim/sulfamethoxazole.

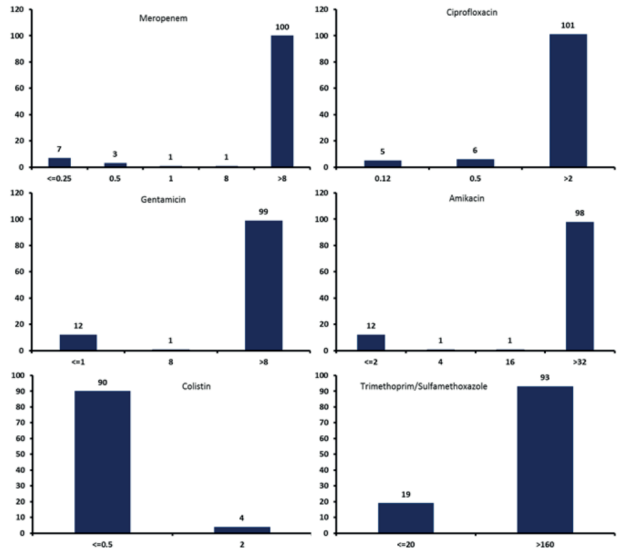

Figure 4. Minimum Inhibitory Concentration (MIC) distributions for meropenem, ciprofloxacin, aminoglycosides, colistin and cotrimoxazole against *Acinetobacter baumannii*. MIC values are expressed in mg/L.

Table 4. Number of isolates of *Acinetobacter baumannii* according to sample.

Sample	N
Rectal swab	42
Urine culture	31
Bronchoalveolar lavage/Bronchial aspirate	22
Blood culture	8
Wound/ulcer swab	8
Biopsy	1
Total	112

Table 5. Number of isolates of *Pseudomonas aeruginosa* according to sample.

Sample	N
Urine culture	211
Rectal swab	154
Wound/ulcer swab	97
Bronchoalveolar lavage/Bronchial aspirate	68
Blood culture	37
Biopsy	26
Ear swab	7
Central venous catheter tip culture	6
Conjunctival swab	1
Total	607

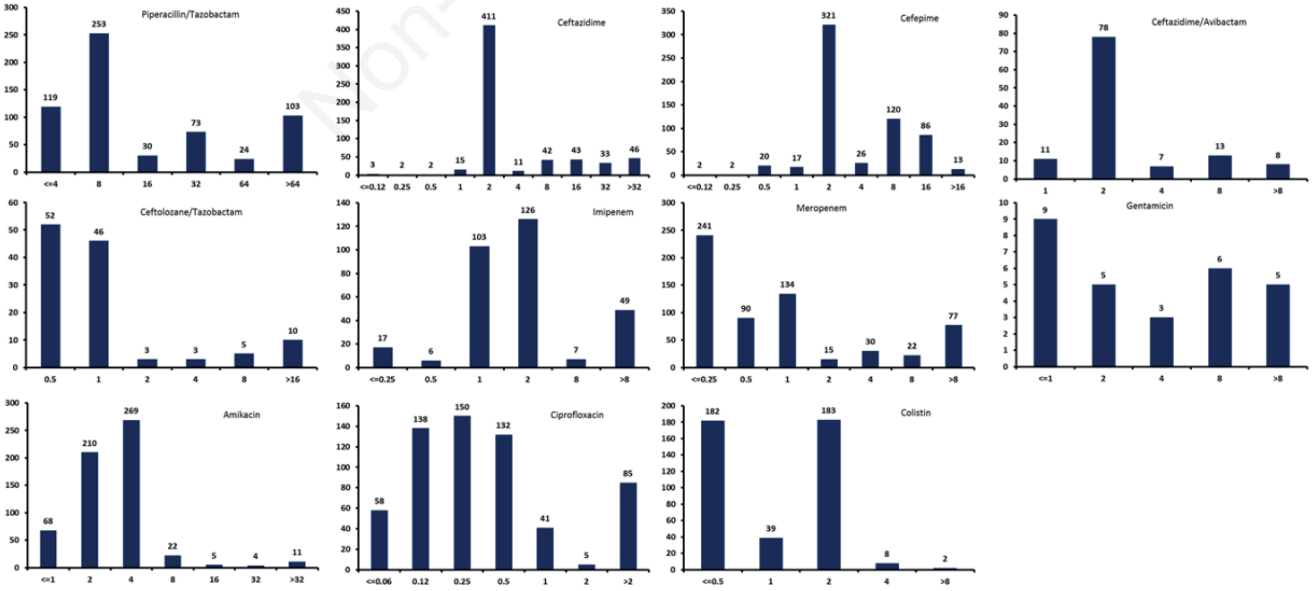

Figure 5. Minimum Inhibitory Concentration (MIC) distributions for main beta-lactam antibiotics, aminoglycosides, ciprofloxacin, and colistin used against *Pseudomonas aeruginosa*. MIC values are expressed in mg/L.

Pseudomonas aeruginosa

A total of 607 isolates of *P. aeruginosa* are described in Table 5. As for *K. pneumoniae* and *A. baumannii*, different Vitek 2® cards were used in relation to the site of infection and so, not all isolates were tested for the same antimicrobials. Figure 5 shows the MIC distributions for the main beta-lactam antibiotics, aminoglycosides, ciprofloxacin and colistin.

Not considering rectal swabs: i) 145/447 (32.4%) isolates were resistant to piperacillin/tazobactam; ii) 59/451 (13.1%) were resistant to both ceftazidime and cefepime; iii) among these 59 isolates, 15/451 (3.3%) were resistant also to imipenem and meropenem; iv) among these 15 isolates, 7 were tested for ceftazidime/avibactam and ceftolozane/tazobactam, 5 (71.4%) had MIC values >8 mg/L for the former and all had MIC values >4 mg/L for the latter, therefore resistant.

Enterobacter species

A total of 311 isolates of *Enterobacter* species are described in Table 6. As for *K. pneumoniae*, *A. baumannii* and *P. aeruginosa*, different Vitek 2® cards were used in relation to the site of infection and so, not all isolates were tested for the same antimicrobials. Figure 6 shows the MIC distributions for the main beta-lactam antibiotics, amikacin, trimethoprim/sulfamethoxazole, ciprofloxacin and colistin.

After exclusion of rectal swabs: i) 72/240 (30%) were resistant to piperacillin/tazobactam; ii) 21/239 (8.8%) were resistant to cefotaxime, ceftazidime and cefepime; iii) among these 21, 2/239 (0.8%) were also resistant to imipenem and meropenem; iv) of these two isolates, one was a Verona Integron–encoded Metallo-β-lactamase (VIM) producer, therefore resistant to ceftazidime/avibactam (MIC >8 mg/L), the other was a KPC producer, susceptible (MIC=8 mg/L).

Table 6. Number of isolates of *Enterobacter species* according to sample.

Sample	N
Urine culture	129
Rectal swab	70
Wound/ulcer swab	35
Blood culture	34
Bronchoalveolar lavage/Bronchial aspirate	30
Biopsy	12
Central venous catheter tip culture	1
Total	311

DISCUSSION

Enterococcus faecium

As in Table 1, more than 50% of the isolates have been recovered from rectal swabs and this explains the high prevalence of high MIC values for glycopeptides observed in the whole sample. No resistant isolates to linezolid nor to tigecycline were recovered, in line with the literature.[9,10]

Staphylococcus aureus

More than 40% of the isolates have been recovered from the skin and soft tissue infections, the most common caused by *S. aureus*.[11] After the exclusion of nasal swabs, around one-fourth of the strains was methicillin-resistant. Almost 80% of the strains had MIC values ≥0.250 mg/L for penicillin G, matching literature data.[9,10] The 97.8% of the isolates had MIC values ≤1 mg/L for ceftaroline, within the range of susceptibility. Nevertheless, none

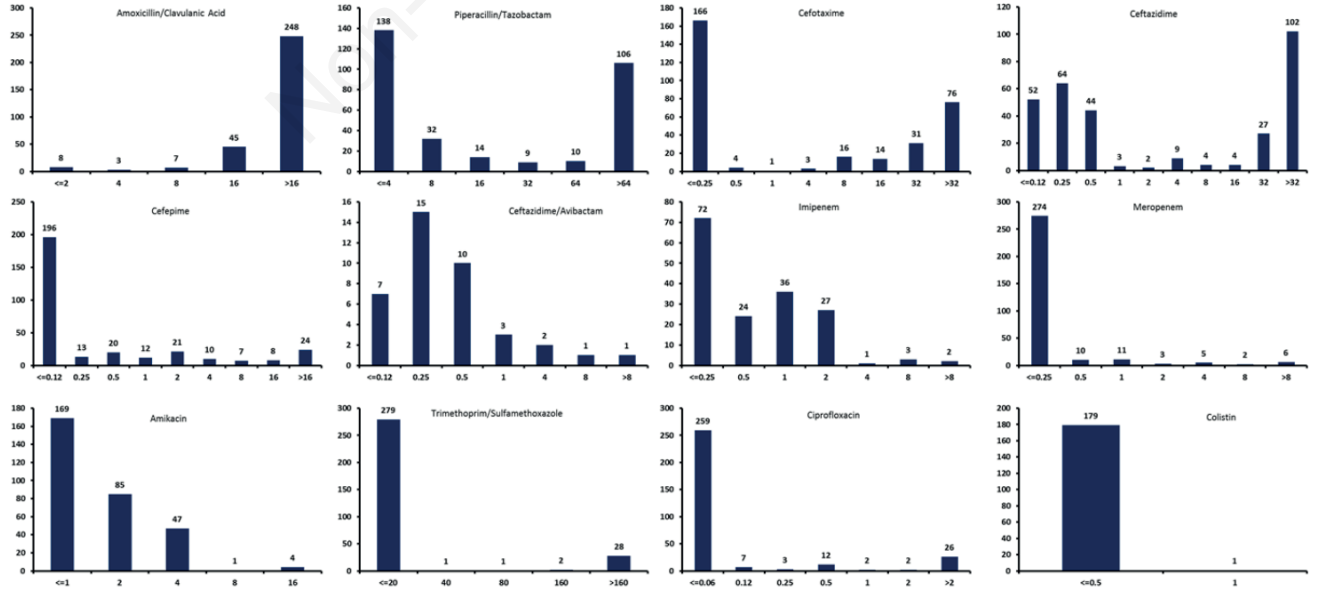

Figure 6. Minimum Inhibitory Concentration (MIC) distributions for main beta-lactam antibiotics, amikacin, cotrimoxazole, ciprofloxacin and colistin used against *Enterobacter species*. MIC values are expressed in mg/L.

of the 11 strains with MIC values of 2 mg/L was recovered from lower respiratory samples, therefore those isolates were considered as "susceptible increased exposure". No resistant strains to glycopeptides, linezolid, daptomycin or tigecyclin were recovered, according to the literature.[9,10] The 96.2% of the isolates had MIC values ≤0.06 for rifampicin. This is probably due to the low antibiotic selection pressure on this drug, rarely used since there is evidence of no benefit compared to standard therapy in patients with bloodstream infection.[12]

Klebsiella pneumoniae

As in Figure 3A about 50% of the isolates had MIC values within the range of resistance for both amoxicillin/clavulanic acid and piperacillin/tazobactam. The same applies to cefotaxime, ceftazidime and cefepime, with proportions of resistance ranging from 47% to 52%. Regarding carbapenems, around one-fourth of the strains had MIC values within the range of resistance. These high MIC values for beta-lactams can be mostly explained by the presence of Extended-Spectrum Beta-Lactamases (ESBL) and carbapenemases found (Figure 3B.) Some of the 8 isolates with MIC values >8 mg/L for ceftazidime/avibactam produced New Delhi Metallo beta lactamase (NDM) and VIM and that explains resistance to that new drug.[13] Other isolates with no such mechanism were stored at -20°C. We deem that the genomic sequencing looking for mutations already described in literature will confirm the resistance to the drug.[14] Genomic sequencing has been indeed recently implemented at the Microbiology Laboratory of SS. Antonio e Biagio e Cesare Arrigo Hospital. As regards aminoglycosides, since in December 2021 EUCAST published a warning against reporting "susceptible" or "susceptible increased exposure" in systemic infections when the MIC values were less than or equal to the ones in brackets described in the breakpoint tables,[15] overall, more than 85% of the isolates (97.6% for amikacin) had MIC values compatible with "isolates without resistance mechanisms". This issue, along with the concerns regarding nephrotoxicity and ototoxicity that require therapeutic drug monitoring [16], possibly explains the high proportion of low MIC values against this class of drugs. Concerning ciprofloxacin and trimethoprim/sulfamethoxazole, around 40% of the isolates showed MIC of resistance. This is conceivable, since both drugs are mainly used to treat Urinary Tract Infections (UTI), more than half of the isolates were recovered from urine cultures and UTI recurrences are frequent, mainly among women.[17] This antibiotic selection pressure can lead to the expansion of resistant clones. Finally, with regard to colistin, less than 2% of the isolates showed MIC values >2 mg/L. All were KPC producers and as for ceftazidime/avibactam-resistant strains, they were stored at -20°C for genomic sequencing to investigate for possible mutations.[18]

Acinetobacter baumannii

From Figure 4, it is clear how around 90% of the isolates showed MIC values of resistance for meropenem, ciprofloxacin and aminoglycosides, whereas 100% showed values ≤2 mg/L for colistin. Therefore, most of the isolates likely represent the spread of the multidrug-resistant clone already described in a previous report[19] and subsequently verified by genomic sequencing.

Pseudomonas aeruginosa

Regarding beta-lactams, the proportions of isolates show MIC values of resistance ranging from one-third for piperacillin/tazobactam to one-fifth or less for ceftazidime, cefepime and carbapenems. Concerning the new anti-pseudomonas association ceftolozane/tazobactam and ceftazidime/avibactam, the resistance rates were 12.6% and 6.8%, respectively. One of the isolates was a VIM producer, and as for *K. pneumoniae*, the resistant strains were stored at -20°C for genomic sequencing.[20,21] Regarding aminoglycosides, the same rule published by EUCAST in December 2021 already described for *K. pneumoniae* also applies to *P. aeruginosa*. As in Figure 5, overall, 97.5% of the isolates had MIC values compatible with "isolates without resistance mechanisms" for amikacin. Nothing to say about gentamicin because no clinical breakpoints are available in EUCAST. Concerning colistin, since EUCAST from 2022 defined MIC values of 4 mg/L in brackets,[10] we can say that more than 99% of the isolates had MIC values compatible with "isolates without resistance mechanisms". With regard to ciprofloxacin, around one-fifth of isolates had MIC values of resistance and since 34.7% of the samples were urine cultures, the same possible effect of antibiotic selection pressure proposed for *K. pneumoniae* can be considered.

Enterobacter species

As in Figure 6, almost all isolates had MIC values of resistance for amoxicillin/clavulanic acid. This was expected, since both *Enterobacter cloacae* complex and *Klebsiella aerogenes* (formerly known as *Enterobacter aerogenes*) are intrinsically resistant to the drug.[22] Regarding piperacillin/tazobactam and the third- and fourth-generation cephalosporins tested, it is clear that the proportions of resistance to piperacillin/tazobactam, cefotaxime and ceftazidime amounted to around 45%, the one for cefepime is of 12.5%. That's imaginable, since *Enterobacter* spp. has inducible chromosomal AmpC, cefepime is a poor substrate for AmpC-type cephalosporinases and AmpC-type enzymes are poorly inhibited by the classical ESBL inhibitors.[23] Regarding the carbapenem resistant strains, three were KPC producers, one was VIM producer. The other two were stored as for *K. pneumoniae* and *P. aeruginosa* for genomic sequencing. The VIM producer was the only strain that showed a MIC value >8 mg/L to ceftazidime/avibactam. Concerning aminoglycosides, 97% of isolates had MIC values overall compatible with "isolates without resistance mechanisms". Less than 10% of resistance rates were observed to ciprofloxacin and trimethoprim/sulfamethoxazole, and no resistance to colistin.

CONCLUSIONS

In this report, we described the antimicrobial resistance rates of ESKAPE pathogens isolated from patients treated between December 2021 and July 2022 at SS. Antonio e Biagio e Cesare Arrigo Hospital, hoping these local prevalence data can be useful as an adjunctive tool to help select antimicrobial therapy.

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