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Nosocomial pneumonia with multi-resistant bacteria in the intensive care units

S Kouara, K Lemhouer *, J Elamouri, M Mahmoud and G Yahyaoui

Central Laboratory for Medical Analysis, Department of Bacteriology, Hassan II University Hospital Fez, Morocco.

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Abstract

A nosocomial pneumonia is a pulmonary infection occurring in a patient after 48 hours of care in a health care facility, it is an infection that is serious because it is often due to multi-resistant germs called multi-resistant bacteria. The objective of our study is to analyze the bacterial ecology of nosocomial pneumopathies in intensive care units and their evolution between January 1st 2021 and December 31st 2021. We conducted a monocentric retrospective study in the microbiology laboratory of the CHU HASSAN II of Fez. We analyzed all the respiratory microbiological diagnostic samples taken during this period (identified germ, sensitivity profile), and collected the demographic characteristics of the associated population. Out of 192 respiratory samples received, 106 were multidrug resistant bacteria (52.6%). The proportions of the different bacterial classes (BGN / CG+) have remained stable over the last 4 years with a predominance of Acinetobacter baumanii with 93%, followed by Klebsiella pneumoniae (4.7%) and Escherichia coli (2.83%)..Bacteria of clinical interest showed increasingly worrying levels of resistance to beta-lactam antibiotics, with the exception of methicillin-resistant Staphylococcus aureus, which remained stable between 2018 and 2021.This work is part of a process of improving antibiotic prescribing practices and analyzing the impact of changes in antibiotic therapy protocols on the ecology of the service.

Keywords: Nosocomial pneumonia; Bacterial ecology; BMR; Intensive care

1 Introduction

Bacterial pneumonia represents the most frequent and severe nosocomial infection in the intensive care unit as it is responsible for a high mortality rate. The diagnosis of bacterial pneumonia is based on a combination of clinical, radiological and microbiological analysis [1]. The rapidity of the implementation of a probabilistic antibiotic treatment conditions the prognosis of the patient, the choice of which depends on several factors such as the result of the direct examination, the notion of colonization, the results of previous bacteriological examinations and of course the bacterial ecology of the department. Among the germs responsible for these respiratory infections we often find Pseudomonas aeruginosa, Staphylococcus aureus, Acinetobacter baumannii, Haemophilus influenzae, Streptococcus pneumonia and Enterobacteria. All these data, as well as the frequency of multiresistance, show the difficulty of choosing an empirical treatment taking into account all these microorganisms [1]. Our study, carried out in the microbiology laboratory of the Hassan II hospital in Fez, aims to describe the bacterial ecology of nosocomial pneumopathies in intensive care units and to draw up the antibiotic resistance profile of the most frequently encountered germs, in order to guide the management of nosocomial infection and the prescription of empirical antibiotic therapy for patients hospitalized in these units.

* Corresponding author: K Lemhouer

2 Material and methods

This is a retrospective, descriptive, observational study, spread over 1 year from January 1, 2021 to December 31, 2021, carried out in the microbiology laboratory of the CHU HASSAN II of Fez, focusing on the microbiological profile of nosocomial pneumopathies with multi-resistant bacteria in the hospital's intensive care units. On respiratory samples of different types (PDP, sputum, BAL) which are processed in the hospital laboratory. The data collected in this study were compared with data collected in previous years, in order to analyze the evolution of bacterial resistance and to evaluate the impact of different antibiotic protocols on bacterial ecology.

Five multi-resistant bacteria were targeted in this study: Acinetobacter baumanii, C3G-resistant Enterobacteriaceae (ESBL), carbapenemase-producing Enterobacteriaceae, ceftazidime- and imipenem-resistant Pseudomonas aeruginosa and methicillin-resistant Staphylococcus aureus (MRSA). All germs diagnosed from intensive care units with a positive culture for any of the above germs were included in the study. Sampling was performed in a nonredundant design. Samples taken for screening for nasal or digestive carriage of resistant germs were excluded. In addition, the sampling was non-probability convenience, all samples meeting the inclusion criteria were recruited.

Data collection was performed by analysis of bacteriology records of respiratory samples. The following information was collected: patient index, age, sex, type of specimen received (PDP, AB, BAL, sputum), culture result and antibiogram result. These data were transcribed on an Excel table and classified according to date.

2.1 Analytical phase

2.1.1 Microscopic examination

The Gram stain result is obtained within a short period of time (usually less than one hour after arrival at the laboratory).Its positive predictive value is better for specimens from sterile sites, but its sensitivity rarely exceeds 50% for specimens contaminated with flora (respiratory). Microscopic examination after May-Grümwald-Giemsa staining is also performed for sputum to assess the number of epithelial cells and leukocytes per microscopic field at x40 magnification. According to the criteria of Bartlett, Murray, and Washington, an optimal specimen should contain <10 epithelial cells and more than 25 polynuclear cells per field. A specimen containing more than 25 epithelial cells per field is considered saliva-contaminated and is therefore not seeded.

2.1.2 Culture

Seeding is performed using a 10 µL calibrated loop. Respiratory samples by star plating, after fluidization and successive dilutions. The choice of medium depends on the type of sample and the suspected bacteria. Ordinary agars (CLED, PBC), selective agars (Chapman for staphylococci, EMB for gram-negative bacilli, CNA for streptococci) and enriched agars (chocolate, fresh blood) are available.

2.1.3 Bacterial identification

The identification of bacterial strains was based on the study of morphological, cultural and biochemical characteristics (fermentation of sugars, reduction of nitrates, search for enzymes such as oxidase, DNAase, catalase...). The precise identification of bacteria (genus and species) was carried out by automated method on Phoenix 100 of Becton Dickinson.

2.1.4 Antibiotic susceptibility testing

For each strain, susceptibility was determined by automated susceptibility testing (Phoenix 100) in liquid medium and standard susceptibility testing by swabbing. According to the Mueller-Hinton agar diffusion method. The reading and interpretation criteria are those of the French Association of Microbiology (CASFM/EUCAST 2020) [2]. Locally prepared Mueller-Hinton (MH) agar is used in the agar diffusion method for bacteria other than slow growing ones. MH-F agar supplemented with 5% defibrinated horse blood and 20 mg/L B-NAD, purchased ready-to-use, is used for streptococcus spp, haemophilus spp and other slow-growing bacteria.

2.1.5 Choice of antibiotics tested

The choice of antibiotics tested was made taking into consideration the standard and complementary lists of CA-SFM / EUCAST 2020.

2.1.6 Detection of ESBL trait

ESBL production by a bacterial strain was confirmed by testing for β-lactam resistance using a qualitative method: the synergy test,Under standard susceptibility testing conditions, a central disk of amoxicillin+ clavulanic acid and a peripheral disk of C3G (Ceftriaxone) placed 3 cm from the central disk were used for qualitative detection of extended-spectrum beta-lactams. The presence of synergy between the two discs detected by the presence of a characteristic "champagne cork" image, confirms the presence of an extended spectrum beta-lactamase.

2.1.7 Carbapenemase detection

Carbapenemase production by a bacterial strain was suspected in the face of decreased susceptibility to Ertapenem (inhibition diameter < 25 mm by agar diffusion test). All suspected strains were subjected to rapid immuno-chromatographic screening.

2.1.8 MRSA Screening

Cefoxitin resistance was tested using a 30 ug cefoxitin disc under standard susceptibility testing conditions.

1. Disc diameter < 22 mm: R 2. FOX diameter > 22 mm: S

Cefoxitin-resistant staphylococcal strains were interpreted as resistant to all beta-lactams.

3 Results

192 respiratory specimens from different patients hospitalized in the ICU of which 106 positive were included in the study. These patients were mainly men, 86 men or 81% and 20 women or 19%. The sex ratio was therefore 4.3. The mean age of our patients was 45 years with extremes ranging from 0 to 92 years.

52.6% of the respiratory specimens received from the intensive care units corresponded to multi-resistant bacteria (MDR) and were distributed as follows: 103 protected distal specimens, 2 bronchoalveolar lavage specimens and one sputum specimen. Table 1.

Table 1 The distribution of germs found in each sampling site

Germs found	PDS	sputum	BAE
AB MR	98	0	1
KP BLSE	2	1	1
E COLI BLSE	3	0	0

These bacteria are dominated by Acinetobacter baumanii and enterobacteria. The distribution of enterobacteria shows a predominance of Klebsiella pneumoniae, representing 4.7%, followed by Escherichia coli with 2.83%.

Table 2 Distribution of BMR isolates

Bacterial isolates	Percentage %
Acinetobacter baumanii multirésistant	93%
Klebsiella pneumonie BLSE	3.77%
Escherichia coli BLSE	2.83%
Klebsiella pneumonie CARBAPENEMASE	0.94%
Staphyloccocus aureus SARM	0%

The distribution of these isolates according to BMR bacterial species is presented in Table 2.In 94% of the pneumopathies were monomicrobial infections and 6% were polymicrobial infections. Figue 1.

During this period, the respiratory samples taken came back sterile in 86 cases, the positive samples (n=106) revealed gram-negative bacteria in 106 cases and no cases of gram-positive cocci (CGP) were found.

The distribution of germs found in the respiratory samples was characterized by a predominance of gram-negative bacteria, in particular Acinetobacter baumanii (n=99), followed by the family of enterobacteria where the genera Escherichia coli and Klebsiella were predominantly represented (n=7). No cases of multi-resistant Pseudomonas aeroginosa were found.

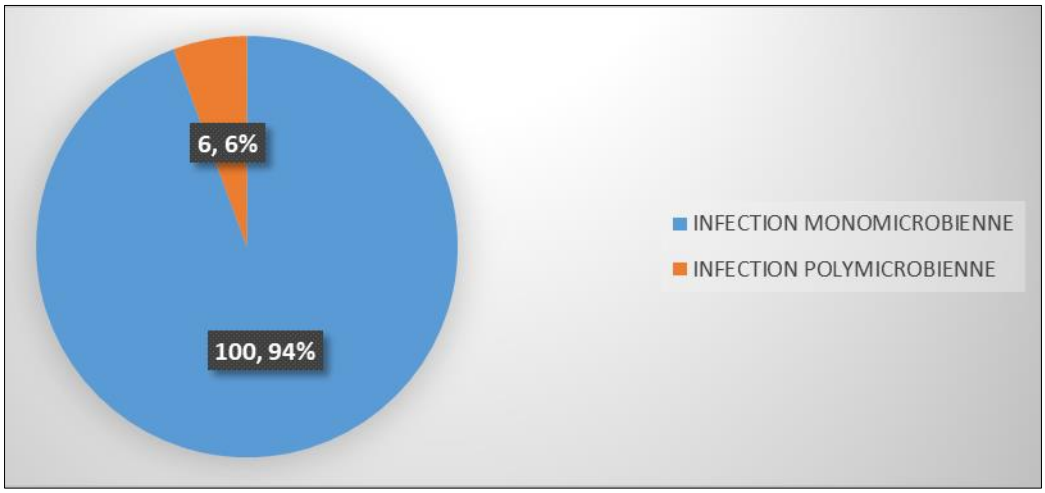

Figure 1 The distribution of single and polybial respiratory infections

Table 3 Percentage of resistance of the main germs to antibiotics

	A. baumanii	***E. coli BLSE***	***Klebsiella BLSE***	***S. aureus***	***P. aeruginosa***
amoxicilline	---	100	100	100	---
Am+Ac.clav	---	95	98	100	---
ceftazidime	100	100	100	100	00
imipenème	100	14.28	12.36	---	00
gentamycine	100	10.2	12.3	7.14	23.53
amikacine	100	00	00	---	5.88
ciprofloxacine	90	14.28	11.2	21.43	5.88
Triméthoprime sulfamétoxazol	---	14.28	12.32	71	82
colistine	00	---	00	---	5.88
ticarcilline	80	---	---	---	58.23
Aztreonam	90	---	---	---	52.13

Acinetobacter baumannii isolates (n=99) showed increased resistance to the majority of antibiotics tested, including aminopenicillins, cephalosporins, imipenem, amikacin and fluoroquinolones. The rate of imipenem-resistant Acinetobacter baumannii was 100%. The most active antibiotic on these isolates was colistin.

All the multiresistant enterobacteria isolated were resistant to aminopenicillins and amoxicillin-clavulanic acid and also to cephalosporins and 2 cases were resistant to fluoro quinolones with only one case of carbapenemase. Our strains were mainly sensitive to amikacin (98% sensitivity) and colistin (100%).

The distribution of ESBL-producing Enterobacteriaceae according to bacterial species shows a predominance of Klebsiella pneumoniae, representing 57%, followed by Escherichia coli with 43%.

No cases of ceftazidime or imipenem resistant Pseudomonas aeruginosa or vancomycin resistant enterococci were isolated in this series.

Cefoxitin-resistant strains of staphylococci were interpreted as resistant to all beta-lactams. In our series, none of the staphylococcus aureus cases were resistant to cefoxitin.

4 Discussion

The bacteria responsible for nosocomial pneumonia vary according to the type of population studied, the length of hospitalization and stay in intensive care, the diagnostic methods used, the existence of previous antibiotic therapy and the duration of mechanical ventilation. In our study, acetobacter baumanii was found in 93% of cases, the same result has been reported by more recent studies, acetobacter baumanii is the most frequently isolated germ [2]. This is consistent with the results of our study.

The current epidemiology is marked by an increasing incidence of polymicrobial pneumonia[13].However, our study showed no difference in terms of epidemiology or progression between patients with polymicrobial pneumonia compared to those with monomicrobial pneumonia. This is in line with several studies including that of Combes et al [2].

At the end of our study on bacterial ecology in the ICU, the objective of which was to establish microbiological documentation of nosocomial respiratory infections in order to guide the empirical prescription of antibiotics. We found that while nosocomial infections cover a variety of clinical contexts, the bacterial etiologies are equally varied.The multidrug-resistant bacteria responsible for nosocomial pneumonia in the ICU are dominated by non-fermenting BGN, notably acinetobacter baumanii (93% of the bacteria isolated), and by fermenting enterobacteria (7% of the bacteria isolated). Gram-positive cocci do not represent any of the isolated bacteria. BGN are represented by Acinetobacter baumanii (93%), Klebsiella pneumoniae (4.7%) and Escherichia coli (2.83%). These data are superposable to those found in other hospitals in the kingdom, and the predominance of Acinetobacter baumanii has also been reported in other national health care centers in Rabat [3], Casablanca [4] and Marrakech [5]. However, these microbiological data contrast with those of European countries where the predominance of Acinetobacter baumanii has not been reported, in favor of enterobacteria and Pseudomonas aeruginosa [6]. According to the European Prevalence of Infection in Intensive Care (EPIIC II) study, which included 1265 intensive care units in 75 different countries, BGN accounted for 62.2% of all included nosocomial infections [7]. Restrepo and Peterson also highlighted this predominance of BGN in a study that analyzed the germs responsible for nosocomial pneumonia in patients enrolled in two large clinical trials in the United States [8].A very worrying increase that makes Acinetobacter baumanii a serious epidemiological problem, justifying the implementation of a surveillance system of the microbial environment of health care institutions and the strict application of hygiene measures.

Bacteria resistant to 3rd generation cephalosporins through the production of ESBLs are a major concern in hospitals because of their epidemic spread, their multiresistance to antibiotics and their participation in the pressure on carbapenems.The control of ESBL-related resistance is essential and is of particular importance, especially since these enzymes are the main vectors of resistance. The control of ESBL resistance is essential and of particular importance, especially as these enzymes are the main vectors of resistance, especially as they are plasmidic and therefore transferable from one strain to another.

According to our study, ESBL-producing Enterobacteriaceae represent 6.6% of the total number of isolated MRB responsible for nosocomial pneumonia. This rate is similar to that reported by other Moroccan studies conducted in Rabat and Marrakech in 2016 [12], and remains even higher than those published by the European Network for Surveillance of Bacterial Resistance to Antibiotics in 2020 [13].These results illustrate the continued progression of ESBL Enterobacteriaceae in our institution and prompt increased vigilance and the implementation of measures to prevent cross-transfer. The increase in the incidence of ESBL is directly implicated in the increase in carbapenem pressure, and thus plays an undeniable role in the appearance of active carbapenemases observed in our hospital, particularly in Klebsiella penumoniae. OXA-48 carbapenemases were predominant in 2018. These β-lactamases have a spectrum as they mainly hydrolyze penicillins and carbapenems [14]. Thus, in the absence of co-production of extended-spectrum β-lactamases, oxa-48 strains can remain susceptible to third-generation cephalosporins (cefotaxime, ceftazidime). However, since 2020, NDM-1 metalloenzyme carbapenemases have become increasingly prevalent and concerning. The severity of these NDM-1 strains is due to several factors: near-permanent multidrug resistance, the size of the organism, and the lack of a surveillance and quality control system. These carbapenemase-producing Enterobacteriaceae infections are difficult to treat and can lead to treatment impasses. The introduction of new antibiotics, such as ceftozolan, and new β-lactamase inhibitors, such as Avibactam, has only partially addressed this

phenomenon. In this context, control of the spread of emerging highly antibiotic-resistant bacteria must be based on a dual strategy of reducing antibiotic prescribing to limit selection pressure and preventing spread from carrier patients. *S. aureus* plays an important role in nosocomial infections.

The development of antistaphylococcal penicillins of the meticillin family was rapidly followed by the emergence of meticillin-resistant staphylococcus aureus (MRSA).In our series, S.A resistance to meticillin is 0%. This rate is much lower than the figures published by other Moroccan hospitals, which show figures varying between 14 and 19%. This resistance to meticillin leads to resistance to all beta-lactams [17].it is determined by the presence of a chromosomal gene (mecA) that codes for an additional PLP, PLP 2a [13].this additional PLP has less affinity for beta-lactams. The therapeutic alternative for these resistant strains remains the use of glycopeptides, the use of which is increasing in parallel with the growing emergence of MRSA.

5 Conclusion

The reading and interpretation of the antibiogram of the most represented bacterial species show that the multi-resistant acinetobacter baumanii is the germ most found in respiratory samples. These results make acinetobacter baumanii a recurrent sanitary problem whose resistance to drugs is more and more threatening, because if colistin remains active on almost all these strains, it is currently the last available therapeutic option.

The results of this work will therefore improve the knowledge on bacterial ecology and on the activity of antibiotics against different pathogens in the intensive care units of the Hassan II Hospital of Fez. Because of the health status and the altered defenses of the patients in intensive care, we insist on the rigorous respect of the rules of hygiene and of the hands in particular as well as on the sensitization of the personnel to the role that the environment of care can hold in the chain of transmission of these micro-organisms.

Finally, we conclude that an efficient fight against these nosocomial infections requires a global prevention strategy which implies a close collaboration between epidemiologists, clinicians, bacteriologists, hygienists and the nursing staff.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest.

Statement of ethical approval

The present research work does not contain any studies performed on animals/humans subjects by any of the authors.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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