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RESEARCH ARTICLE

# Institutional vigilance of antimicrobial susceptibility in pathogens of clinical interest

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## ABSTRACT

**Background.** The increased resistance of microorganisms to antibiotics has led to an increase in morbidity and mortality from infections, increased use of antibiotics and excessive hospitalization costs. Therefore, the aim of this study was to describe the frequency of pathogens and bacterial susceptibility patterns in cultures of blood, urine and other bodily fluids in a tertiary care pediatric hospital. We also aimed to determine the patterns of resistance in pathogens of clinical interest isolated in blood, urine and other sterile liquids in a pediatric teaching center and third-level hospital.

**Methods.** The Institutional Antimicrobial Surveillance Program was established to monitor the predominant pathogens and antimicrobial susceptibility patterns of infections such as bacteremia, pneumonia and urinary infections. The species of each isolate was determined according to routine methodology and Vitek system from January 2010 to June 2011. Antimicrobial agents and susceptibility testing were determined using the Vitek 2XL according to the Clinical and Laboratory Standards Institute.

**Results.** We recovered 7,708 isolates from 27,209 cultures (28.3%). Gram negative represented 52.7%. A rank order showed coagulase-negative *Staphylococcus*, *Escherichia coli, Enterococcus* spp., *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae* and others. The antimicrobial susceptibility of the most frequently encountered pathogens was variable. *E. coli* showed the highest resistance to trimethoprim-sulfamethoxazole and ampicillin-sulbactam (74 and 68%, respectively) finding the best option to be nitrofurantoin and imipenem with 84 and 100% sensitivity, respectively. *Enterococcus faecium* resistance was 58% vancomycin, and *Streptococcus pneumoniae* showed 100% sensitivity to vancomycin.

**Conclusions.** This study emphasizes the problem of resistance and the needs to select an appropriate broad-spectrum empirical regimen guided by the knowledge of pathogen occurrences and local/regional/global resistance patterns. Such practices require the interrelation between clinical microbiology laboratories and hospital pharmacies.

**Key words:** antimicrobial susceptibility, multidrug-resistant Gram-negative, Gram-positive resistant patterns.

## INTRODUCTION

Resistance to antibiotics is a public health problem that increases day by day. For this reason, it has been necessary to generate monitoring networks worldwide.[1-5] The increased resistance of microorganisms to antibiotics has led to an increase in morbidity and mortality from infection, at the time of hospitalization, in the use of antibiotics and increased costs of hospitalization.[6]

Increased resistance to antibiotics is due to, among other factors, their indiscriminate use, which causes the occurrence of different single or multiple mechanisms of resis-



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tance in the pathogens. The presence of bacterial pathogens in blood, spinal fluid, pleura, peritoneum and other locations is a significant cause of morbidity and mortality.[7-11]

Gram-positive bacteria such as *S. aureus*, coagulase-negative *Staphylococcus* (CNS), *Enterococcus* spp., *Streptococcus viridans* and *Streptococcus pneumonia* occupy a defined place in infectious diseases, which is complicated by bacterial resistance against the routinely used antibiotics.[12-15]

In recent years, new resistance mechanisms have been recognized. It is not surprising to now consider pathogens with multiple resistances or even extremely resistant to more than a dozen drugs.[10-18] It has been shown that early initiation of appropriate antibiotics is critical for reducing morbidity and mortality in critically ill patients. Antibiotic initiation is often empirical, requiring knowledge of its pathogenic potential as well as the usual susceptibility patterns. Under these conditions, the resistance makes empirical selection of one or more drugs difficult.

The goal of the clinician, when requesting a pattern of susceptibility, is to predict how a bacterial strain behaves when confronted or challenged to an administered antibiotic. A sensitivity result will determine that the bacteria will be eliminated and that the patient will respond to treatment with the antibiotic. Resistance to a particular antibiotic will indicate that the infectious process will continue and that the bacteria will not be eliminated. Hospital Infantil México Federico Gomez (HIMFG) is a center of concentration for patients with highly complex diseases where 60–65% of the population suffers from some type of neoplasm (mainly leukemias and lymphomas) and receives chemotherapy. Due to neutropenia, patients develop respiratory infections (pneumonia), urinary tract infections, diarrhea, neutropenic colitis, and septic shock as associated problems.

Therefore, knowing the susceptibility patterns allows the clinician to select the most appropriate antibiotic, considering the clinical and biological factors of the patient.

The purpose of this report is to describe the frequency of pathogens isolated and characterized along with their antimicrobial susceptibility profile.



## SUBJECTS AND METHODS

The study protocol was approved by the Institutional Research Committee. We studied the cultures obtained from HIMFG patients, a pediatric tertiary care institution, from January 2010 to June 2011. We identified and characterized microorganisms isolated from blood, urine and other bodily fluids. We studied the susceptibility pattern against different antimicrobial drugs. Only the initial strain of each culture was included.

Cultures of blood and other fluids such as pleural, pericardial and peritoneal were inoculated in culture flasks (BacT/ALERT PF, bioMèrieux). Control of the functionality of the flasks was performed by inoculating bacterial strains of identity and concentrations as referred to from the American Type Culture Collection (ATCC). Urine samples were considered for the study of a single urine culture in which a germ was isolated with $10^3$-$10^5$ colony-forming units (CFU)/ml.[19]

For identification of bacterial strains isolated from patients, we used basic manual testing of conventional identification such as colony morphology, Gram stain, catalase and oxidase[20] as well as the procedure of identification by the automated Vitek 2XL (bioMèrieux).

Susceptibility tests to antibiotics were determined in accordance with the guidelines of the Clinical Laboratory Standards Institute (CLSI)[21] using the automated system (Vitek 2XL bioMèrieux) and Kirby-Bauer method to verify methicillin-resistant staphylococci (MRSA). An oxacillin disk (1 g) was used in Mueller-Hinton plates containing 2% sodium chloride and cefoxitin discs (30 mg) in Mueller-Hinton plates. *S. aureus* strains ATCC-29213 and ATCC-BAA-1026 were used as a reference.

In the case of *S. pneumoniae*, the cut-off levels for strains of meningeal and non-meningeal origin were used according to the CLSI. Susceptible or resistant strains were determined according to the minimum inhibitory concentration as per the CLSI parameters.[21] Bacteria in an intermediate range were assumed to have reduced sensitivity.

To define a case of nosocomial infection, we considered the 72 h after admission, during the hospitalization period and 72 h after discharge. Cases of community-acquired infections were patients who were admitted to the HIMFG with clinical data associated with infection and indeterminate were cases where it was not possible to document the period of onset of infection.

## RESULTS

There were 7708 microorganisms isolated from 27,209 samples (28.3%) of pediatric patients: 74.6% from hospitalizations and emergency care and 25.4% from outpatient services from January 2010 to June 2011. Of the positive cultures, 44%

were obtained from blood, 46% from urine, 6% from different body fluids (pleural, pericardial and peritoneal) and 4% from cerebrospinal fluid (CSF). The recovery corresponded to 16% in blood cultures, 21% in urine cultures, 22% in body fluids and 8% in CSF (Table 1). It was considered, according to the established criteria, that the origin of the infection was nosocomial in 1772 patients, community-acquired in 3085 patients and in 2851 patients it was not possible to accurately document the origin of the source of infection.

Table 2 lists, in order of frequency, microorganisms identified in 7708 positive cultures. Those less common, *Acinetobacter*, *Kluyvera ascorbata* and *Aeromonas* sp., are not presented. The first place is occupied by the coagulase-negative *Staphylococcus* (CNS) group, with 25.8% frequency. Within this group, *S. epidermidis* accounted for more than 85% and *S. hominis, S. haemolyticus* and *S. auricularis* for the remaining 15%.

*E. coli* was identified in 1421 urine cultures (80%) and 355 in blood or other bodily fluids. Other pathogens found from urinary tract infections were *Klebsiella* spp., *E. faecalis* and *E. faecium*. There were 1928 blood cultures identified: in order of frequency they represented CNS, *S. aureus, K. pneumoniae, P. aeruginosa, E. coli, Enterococcus* spp. and *S. pneumoniae*.

Table 3 describes the antimicrobial susceptibility of the most frequent Gram-negative pathogens. *E. coli* strains had a higher resistance to trimethoprim–sulfamethoxazole and even to ampicillin–sulbactam (74 and 68%, respectively). Minor resistances were found for amoxicillin–clavulanate (27%) and cefuroxime 24% (data not shown). Resistance to aminoglycosides such as gentamicin, was 31%. For the group of third-generation cephalosporins it was 40%. The best options for sensitivity were for piperacillin–tazobactam (72%), nitrofurantoin (84%) and imipenem (100%).

Strains of *P. aeruginosa* show a resistance of between 23 and 27% to drugs used widely in hospitals such as cefepime and imipenem. The highest resistance found was to trimethoprim–sulfamethoxazole (94%). Antibiotics with greatest susceptibility to this pathogen were gentamicin (64%), imipenem (73%), ceftazidime (75%), ciprofloxacin (82%) and piperacillin/tazobactam (83%).

For *K. oxytoca*, increased susceptibility was for ceftazidime, ceftriaxone and cefepime, with 84%. For *K. pneumoniae*, antibiotics with the greatest susceptibility were ciprofloxacin (94%), levofloxacin (97%) and imipenem (100%), and with much lower numbers to other drugs (between 30 and 40%).

For *E. cloacae*, 100% susceptibility was found for imipenem and 90% for ciprofloxacin, cefepime and levofloxacin. Resistance against ceftriaxone and ceftazidime was 33 and 36%, respectively. The specific antimicrobial activity against some of the Gram-positive specimens is shown in Table 4. *E. faecalis* resulted with significant resistance to gentamicin (53%) and highly sensitive to vancomycin and other β-lactams.

*E. faecium* resistance is manifested with β-lactams, aminoglycosides (gentamicin) and, significantly, vancomycin (58%). Total susceptibility is shown to linezolid and tigecycline. *S. pneumoniae* was resistant to penicillin (25%) and erythromycin (49%) and was 100% sensitive to vancomycin, linezolid and moxifloxacin. It presented acceptable susceptibilities to 3rd and 4th generation cephalosporins. There were 67 strains of *S. pneumonia* identified, of which 97% were not associated with meningitis and were isolated from blood, pleural and peritoneal fluid cultures.

For the 48 strains of *S. viridans*, resistance was 70% for penicillin, 62% for erythromycin, 43% for tetracycline and 42% for cefotaxime. The highest susceptibility for *S. viridans* was 83% for clindamycin and 100% for vancomycin.

**Table 1.** Distribution of the cultures and percentage of recuperation



| *Type of culture* | *Total cultures (27,209)* | *Positive cultures* | *Microbiological recuperation (%)* |
|---|---|---|---|
| Hemocultures | 12,071 | 1928 | 16.0 |
| Urocultures | 12,350 | 2656 | 21.5 |
| CSF | 1669 | 148 | 8.8 |
| Various fluids (pleural, pericardial and peritoneal) | 1119 | 250 | 22.0 |

CSF, cerebrospinal fluid.

Table 5 analyzes the susceptibility and resistance of *S. aureus* and coagulase-negative *Staphylococcus* (CNS) according to their susceptibility to methicillin. MRSA was virtually resistant to all β-lactams. It had variable but significant resistance to erythromycin, clindamycin, ciprofloxacin, and 23% for moxifloxacin. It was highly sensitive to trimethoprim–sulfamethoxazole, gentamicin, rifampicin, linezolid, tetracycline, tigecycline and vancomycin

Methicillin-sensitive *S. aureus* (MSSA) showed significant resistance to penicillin (92%). For the remainder of the drugs, susceptibility varied between 80 and 100%, with oxacillin, vancomycin, linezolid and tigecycline noteworthy with 100% susceptibility. MRCNS strains were resistant to all β-lactams. They had variable resistance to other antimicrobial drugs such as clindamycin (82%), erythromycin (85%) and ciprofloxacin (69%), trimethoprim–sulfamethoxazole (64%), gentamicin (65%) and were highly sensitive to linezolid, tigecycline and vancomycin. Of the 536 strains of *S. aureus*, 51.67% were resistant to oxacillin.

**Table 2.** Frequency of pathogens isolated from different sources (January 2010-June 2011)

| *Organism* | *No. isolates 2010-2011* | *Frequency (%)* |
|---|---|---|
| CNS | 1995 | 25.88 |
| *Escherichia coli* | 1776 | 23.04 |
| *Enterococcus* spp. | 808 | 10.48 |
| *Staphylococcus aureus* | 760 | 9.86 |
| *Pseudomonas aeruginosa* | 716 | 9.29 |
| *Klebsiella pneumoniae* | 746 | 9.68 |
| *Enterobacter* spp. | 301 | 3.91 |
| *Proteus mirabilis* | 122 | 1.58 |
| *Stenotrophomonas maltophilia* | 97 | 1.26 |
| *Morganella morganii* | 90 | 1.17 |
| *Serratia marcescens* | 68 | 0.88 |
| *Streptococcus pneumoniae* | 76 | 0.99 |
| *Klebsiella oxytoca* | 54 | 0.70 |
| *Salmonella* spp. | 57 | 0.74 |
| *Citrobacter freundii* | 42 | 0.54 |
| Total | 7708 | 100.00 |

CNS, coagulase-negative *Staphylococcus.*

## DISCUSSION

Much of what we know about the global epidemiology of bacterial resistance and even of multiple resistance comes from large surveillance databases organized by the pharmaceutical industry and reports of outbreaks identified in microbiology laboratories, as well as series or data submitted by public health groups, either nationally or internationally.[2,5,7-10,12,15,21]

Because of the frequency of resistance among hospital pathogens such as MRSA and vancomycin-resistant *Enterococcus* (VRE), from the 1990s the focus was on Gram-positive microorganisms. That made it possible for new drugs to be available in the market for its treatment.

**Table 3.** Activity of various antimicrobials in the most frequent Gram-negative organisms

| | *Susceptibility of microorganisms* | | | | |
|---|---|---|---|---|---|
| *Antibiotics* | *Escherichia coli (n =1769) %R* | *Klebsiella oxytoca (n =54) %R* | *Klebsiella pneumoniae (n =735) %R* | *Pseudomonas aeruginosa (n =696) %R* | *Enterobacter cloacae (n =243) %R* |
| Ampicillin/Sulbactam | 68 | 33 | 69 | 100 | 60 |
| Piperacillin/Tazobactam | 28 | 9 | 57 | 17 | 30 |
| Ceftazidime | 40 | 16 | 69 | 25 | 36 |
| Ceftriaxone | 40 | 16 | 69 | NP | 33 |
| Cefepime | 39 | 16 | 68 | 23 | 10 |
| Amikacin | 4 | 12 | 32 | 31 | 11 |
| Imipenem | 0 | 0 | 0 | 27 | 0 |
| Gentamicin | 31 | 11 | 64 | 36 | 12 |
| Ciprofloxacin | 47 | 1 | 6 | 18 | 10 |
| Levofloxacin | 45 | 1 | 3 | 24 | 10 |
| Nitrofurantoin | 16 | 5 | 47 | 100 | 36 |
| Trimethoprim/Sulfamethoxazole | 74 | 21 | 42 | 94 | 25 |

%R, percentage of resistant strains; NP, no present clinical activity.

In recent years, the problem of antimicrobial resistance has become more complex. Gram-negative bacteria are *K. pneumoniae, A. baumannii, P. aeruginosa*, *E. coli* and other enterobacterias.[10,11,16,17]

Gram-positive and -negative bacteria show novel mechanisms of resistance to one or more antibiotics, which complicates the initial selection of antimicrobial therapy that, in patients with serious infections, should be empirically initiated as soon as possible.

**Table 4.** Activity of various antimicrobials against *Enterococcus* spp.

| | *Susceptibility of microorganisms* | |
|---|---|---|
| *Antibiotic* | *Enterococcus faecalis (%R)* | *Enterococcus faecium (%R)* |
| Penicillin | 7 | 96 |
| Ampicillin | 5 | 96 |
| Gentamicin | 53 | 49 |
| Ciprofloxacin | 37 | 57 |
| Levofloxacin | 38 | 78 |
| Moxifloxacin | 49 | 76 |
| Tigecicline | 0 | 0 |
| Vancomycin | 1 | 58 |
| Linezolid | 0 | 0 |
| Nitrofurantoin | 4 | 83 |

%R, percentage of resistant strains.

**Table 5.** Antimicrobial activity in *Staphylococcus aureus* and coagulase-negative *Staphylococcus*

| | *Susceptibility of the microorganisms* | | | |
|---|---|---|---|---|
| *No. of strains (n)* | *277 MRSA* | *259 MSSA* | *1,712 MRCNS* | *295 MSCNS* |
| *Antibiotics (%R)* | | | | |
| Penicillin | 97 | 92 | 100 | 70 |
| Oxacillin | 100 | 0 | 100 | 0 |
| Gentamicin | 2 | 1 | 65 | 2 |
| Ciprofloxacin | 92 | 7 | 69 | 4 |
| Levofloxacin | 86 | 3 | 67 | 5 |
| Moxifloxacin | 23 | 0 | 7 | 0 |
| Trimethoprim/Sulfamethoxazole | 1 | 1 | 64 | 9 |
| Tigecycline | 0 | 0 | 0 | 0 |
| Vancomycin | 0 | 0 | 0 | 0 |
| Nitrofurantoin | 1 | 0 | 12 | 0 |
| Linezolid | 0 | 0 | 0 | 0 |
| Clindamycin | 93 | 18 | 82 | 16 |

MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*; MRCNS: methicillin-resistant coagulase-negative *Staphylococcus*; MSCNS: methicillin-sensitive coagulase-negative *Staphylococcus*; %R, percentage of resistant strains.

Surveillance programs of bacterial resistance demonstrate that there is wide geographical variation in the prevalence of bacterial resistance. Therefore, studies such as the present one support local decisions for prescribing antibiotics. The main etiology in patients with sepsis and septic shock according to blood culture is primarily to Gram-positive cocci such as *Staphylococcus* spp., *Enterococcus* spp., *S. pneumoniae* and some strains of *S. viridans*, a situation similar to that reported in other databases.[2-5,22]

Strains of *S. aureus* and CNS sensitive to methicillin are 100% sensitive to oxacillin, which allows for that drug to be considered as an initial primary choice for treatment. However, in other series there is concern about the steady increase in the resistance of *Staphylococcus* spp. to oxacillin.[2-5] However, when it comes to nosocomial infections, the problem is complicated by the multiple resistances to various drugs, which justifies the use of a glycopeptide associated with a β-lactam. According to data from our environment, at least one in five patients with positive blood cultures had pneumonia, 65% had septicemia and 15% were with septic shock. This situation complicates the choice of antibiotic treatment scheme and supports, for example, the scheme of combining glycopeptides and aminoglycosides with third- or fourth-generation β-lactams. The justification for these associations is the presence of gram-negative strains, mainly *E. coli, K. pneumoniae, Enterobacter* spp., *P. aeruginosa* and other enterobacteria. These bacteria all have different unique and/or multiple resistance against β-lactams,[3,4,10,16,17] aminoglycosides, glycopeptides and other antimicrobial agents.

For this reason, there is currently a need (sometimes urgent) to know the descriptors that classify these Gram-negative bacteria as bacteria with broad-spectrum β-lactamase production, resistance to the presence of AmpC cephalosporinases, β-lactamases, serine, and metallocarbapenemases and different mechanisms for resistance against quinolones.[10,16,17]

There are several noteworthy points in the results of this paper, mainly based on the percentage of resistance of certain pathogens. For *Staphylococcus* spp., resistance to oxacillin, clindamycin, erythromycin, and ciprofloxacin continues to increase, and there is no resistance to vancomycin, linezolid or tigecycline. We do not have evidence that some strains may have an increase in the minimum inhibitory concentrations ≥2 μg/ml against vancomycin.[23]

For *E. faecalis*, in general the resistance has not changed. However, there is a sustained increase in *E. faecium* resistant to vancomycin (58%), which represents the intermediate figures mentioned in the literature.[12]

Resistance to other antibiotics such as gentamicin (49%) and tetracycline (44%) is important because this pathogen is related to serious systemic infections, which complicates the decision of the antimicrobial scheme to be selected for each patient. *S. pneumoniae*[15] certainly continues to show high resistance to penicillin and erythromycin. Most of our isolates were not from meningitis. Susceptibility to broad spectrum amoxicillin and cephalosporins was higher at 90% and vancomycin continues to be used for multiresistant strains. The Regional Report from SIREVA 2010 presents the geographical variation in resistance to different antimicrobial drugs of this pathogen in systemic infections.[24]

*E. coli* is the most common Gram-negative isolate and represents the most common bacteria in urinary tract infections (80%). This is different from that reported by Mathai et al. and in other series published.[25,26] The lower participation of *E. coli* may be due to the fact that the majority of patients with urinary tract infection also had neutropenia, fever and urinary focus of infection. This may be the same explanation for the increased frequency of other bacteria such as *Klebsiella* spp., *Pseudomonas* spp., *Enterobacter* spp. and *Proteus* spp. Many of these bacteria were also isolated from blood cultures of patients with sepsis and septic shock with elevated patterns of resistance to what is reported in the literature in recent years, including quinolones.[10,16,17,27] *P. aeruginosa* was most frequently identified in pleural fluid cultures, *S. aureus* in synovial fluid and *E. coli, S. epidermidis* and *K. pneumonia* in peritoneal fluid mixtures. Resistance to third- and fourth-generation cephalosporins is ~70% for *K. pneumoniae* and 40% for *E. coli*, both important producers of broad-spectrum β-lactamase, AmpC cephalosporinases, and serine-metallocarbapenemases[10,16,17] which, as a result, significantly reduces the possibilities of antimicrobial regimen selection. This situation is similar to what happens with non-fermenting bacilli such as *P. aeruginosa*, which complicate the resistance to many other drugs such as aminoglycosides and piperacillin-tazobactam. It is important to consider that surveillance studies of antimicrobial resistance have recognized limitations and are repeated in different studies. This work was not the exception.[1]

Finally, knowing the information, in general, of the strains isolated from clinical samples and resistance patterns helped to confirm that the emergence of antimicrobial resistance is a real problem in the care of patients with serious infections. Therefore, clinicians should consider antimicrobial resistance as a public health problem, which is one of the greatest future challenges.

On the other hand, taking into account the limitations of our study, an antimicrobial surveillance network should be strengthened within our institution, establish trends in real time, correlate the information with the data of nosocomial infections and analyze control measures and consumption of antimicrobials.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this study.

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