# Antimicrobial resistance profile of microbial pathogens isolated from hospitals of Colatina-ES in the period of 2008-2010

*Perfil de resistência microbiana dos principais patógenos isolados em amostras provenientes de hospitais de Colatina - ES no Período de 2008 - 2010*

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**Resumo:** A resistência bacteriana é um mecanismo usado pelas bactérias para interferir na ação dos antibióticos e vem crescendo consideravelmente, se tornando um grande problema de saúde pública. O presente estudo avaliou o perfil de resistência de cepas de *Staphylococcus aureus, Pseudomonas aeruginosa e Escherichia coli* e a suscetibilidade das mesmas à alguns antimicrobianos. Para isso realizou-se uma pesquisa quantitativa utilizando os resultados de antibiogramas constantes nos livros de registro microbiologia do laboratório LAC - Santa Maria, situado em Colatina - ES. As amostras foram coletadas rotineiramente de pacientes internados em dois hospitais (Santa Maria e São José) e examinados pelo laboratório. No livro de registro obteve-se um total de 446 isolados microbianos identificados, dos quais 277 cepas foram identificadas como: *S. aureus* (36,5%), *P. aeruginosa* (32,5%) e *E. coli* (31%), os 169 restantes englobam os outros micro-organismos. Quanto ao perfil de resistência e sensibilidade, as cepas de *P. aeruginosa* foram mais sensíveis ao antibiótico sulbactam, *E. Coli* à ampicilina e ácido nalidíxico, e *Staphylococcus* à amicacina e ceftriaxone. Relaciona-se o maior isolamento de *Stapylococcus, P. aeruginosa e E. coli* provavelmente por serem micro-organismos encontrados colonizando ambientes hospitalares e a comunidade com grande potencial de disseminação. Quanto ao perfil de resistência se torna útil na seleção de drogas especificas para determinados micro-organismo.

**Palavras-chave:** Resistência. Antibióticos. Micro-organismos.

**Abstract:** Resistance mechanisms are used by bacteria to interfere with the action of antimicrobial drugs, and they have grown considerably in the latest years, becoming an important public health problem. The present study evaluated the resistance and susceptibility profiles of clinical strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*, in two hospitals in the city of Colatina (ES). Samples were isolated from tips of central venous catheters, tracheal secretions, blood culture, urine, tissue culture, abscesses, purulent secretions, maxillary sinus secretions, pus, pleural liquid, sputum and faeces from patients. The antimicrobial susceptibility tests were performed by the disk diffusion method. From a total of 446 microbial isolates, 277 strains were identified as *S. aureus* (36.5%), *P. aeruginosa* (32.5%) and *E. coli* (31%), and the remaining 169 comprised other microorganisms. *P. aeruginosa* strains were sensitive to sulbactam, *E. coli* strains to ampicillin and nalidixic acid, and *S. aureus* strains to amikacin and ceftriaxone. The significant isolation of *S. aureus*, *P. aeruginosa* and *E. coli* strains is probably because they are extensively found colonizing hospital settings and the community, with great potential for dissemination. *Conclusion:* Considering the scarcity of data regarding patients with varied infections, our findings become even more relevant. The impact of inappropriate antimicrobial therapy and drug resistant strains are very important and should, therefore, be verified by further molecular studies.

**Keywords:** Resistance, Antimicrobials, Microorganisms.

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## Introduction

Varied resistance mechanisms are used by bacteria to interfere with the action of antimicrobial drugs, and due to the inappropriate use of antimicrobials in the latest years, increased levels of resistance acquired by bacteria can be found in both hospital settings and the environment, creating, therefore, a need of knowing the resistance and susceptibility profiles of such microorganisms to antimicrobial drugs, what becomes even more important when designing effective drug therapy. Several studies have suggested that some of these bacteria can be present in the environment and may be highly pathogenic to immunocompromised individuals and cause relevant infections, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* [1,2]

Intrinsic and acquired antimicrobial resistance profiles have been studied: intrinsic resistance is associated to specific bacterial factors, such as lack of a binding site for a given drug. Acquired resistance, as the name implies, is described as acquired resistance by microorganisms, for instance, during drug therapy discontinuation or due to limitations regarding intracellular access of certain drugs [3,4,5].

Hospital infections are defined as infections acquired after admission of the patient and manifested during hospitalization or after discharge even when it can be related to hospitalization or hospital procedures. It is estimated that every 10 hospitalized patients will have infection after hospital admission. Hospital infections are caused by various microbial agents and their primary pathway of dissemination involves the human being. These infections can spread over the organism, causing infectious diseases of difficult control and eradication [6,7].

The first government intervention in Brazil occurred in 1983 by the Ministry of Health, through decree No. 196 of June 24, establishing the Committee on Hospital Infection Control in all hospitals of the country. The same ordinance also describes criteria for identification, diagnosis and standards for selection of germicidal agents. Other ordinances were later released and also conferences on hospital infections were performed,

but the most recent is the ordinance of 1998, and the last action of the Ministry of Health on the National Program of Hospital Infection, which became subjected to the Brazilian Health Surveillance Agency (ANVISA) [8,9].

Hospital infections, both in Brazil and in the world, become a major public health problem, considering different paramenters such as the emergence of antimicrobial resistant microorganisms and invasive procedures. A survey conducted in Brazil in 1995 showed that the rate of infections in Brazilian hospitals is around 15.5 %. It is estimated that in developed countries, approximately 5 % of hospitalized patients have infection during hospitalization and annually, values between 50.000 and 100.000 deaths are associated with hospital infections [8,9,10].

Therefore, this study aimed to investigate the resistance profile and microbial susceptibility of the main pathogens isolated of inpatients in two hospitals from Colatina city, ES, Brazil, in the period of 2008-2010. Antimicrobial disk diffusion method was used as recommended by the CLSI. Given that most of antimicrobial susceptibility studies are performed for single pathogens, and the scarcity of information from this region of the country, our data becomes even more relevant.

**Methodology**

This study was carried out in LAC-Santa Maria clinical laboratory (Colatina city, ES, Brazil), which is responsible for Casa de Saúde Santa Maria and São José Maternity Hospitals. Data such as name, age, sex, address, clinical picture, prescriber in charge or any other personal feature of patients was not assessed, and ethical approval was obtained from the Laboratory prior to commencement of this study. The analysis included 446 isolates over a period of March 2008 to April 2010, and only one strain per patient was chosen.

The isolation and identification procedures were performed according to the conventional methods of bacteriology, and the sources of the isolates were: tip of central venous catheters, tracheal secretions, blood culture, urine, tissue culture, abscesses, purulent secretions, maxillary sinus secretions, pus, pleural liquid, sputum and faeces.

The antimicrobial susceptibility tests were performed through the Kirby–Bauer method with the disks (Sensifar) described in table 1. CLSI breakpoints were used as the interpretative criteria[11].

## Results

From a total of 446 microbial isolates, 277 microbial isolates (62%) corresponded to the clinically important species *Pseudomonas aeruginosa* (32%), *Escherichia coli* (31%) and *Staphylococcus aureus* (36%). Among *S.aureus* isolates, 22.7% were MRSA or ORSA.

Most of the isolates were collected from urine samples, followed by abscesses, tracheal secretions and catheter tips (figure 1). *S. aureus* was the most frequent microorganism of the study, corresponding to 36% of all microorganisms of each source.

The antimicrobial susceptibility tests indicated ceftriaxone (11.87%), sulbactan (11.13), ciprofloxacin (8.71%), levofloxacin (8.53%) and gentamicin (8.53%) as the most resisted drugs for *P.aeruginosa* strains; ampicillin (14.33%), piperacilin (8.6%), nalidixic acid (8.3%) and cefaloridin (8.0%) as the most resisted drugs for *E.coli* strains, and ampicillin (14.33%), piperacilin (8.6%) nalidixic acid (8.3%), amikacin (7.1%) and ceftriaxone (7.1%) as the most resisted drugs for *S.aureus* strains (figures 2a, b, c).

Table 1 - Antimicrobial disks used in assays

| **Antimicrobial Drugs** | | |
|---|---|---|
| Nalidixic acid (30 µg) | Ciprofloxacin (5 µg) | Piperacilin-Tazobactan(100/10 µg) |
| Amikacin (30 µg) | Clindamycin (2 µg) | Polymyxin B (300 U) |
| Ampicillin (10 µg) | Chloranphenicol (30 µg) | Sulbactan (10 µg) |
| Cefaclor (30 µg) | Gentamicin (10 µg) | Sulfazotrin (25 µg) |
| Cephaloridine (30 µg) | Imipenen (10 µg) | Tircacilin (75 µg) |
| Cefepime (30 µg) | Levofloxacin (5 µg) | Azetreonan (30 µg) |
| Cefotaxime (30 µg) | Nitrofurantoin (300 µg) | Tobramycin (10 µg) |
| Erythromycin (15 µg) | Norfloxacin (10 µg) | Tetracycline (30 µg) |
| Cefuroxime (30 µg) | Oxacillin (1 µg) | Piperacillin (100 µg) |
| Ceftazidime (30 µg) | Penicillin (6 µg) | Ceftriaxone (30 µg) |
| Tircacilin / Clavulanic Acid (75/10 µg) | Cefotaxime / Clavulanic Acid (30/10 µg) | Sulfamethoxazole- Trimethroprim (23.75/1.25 µg) |

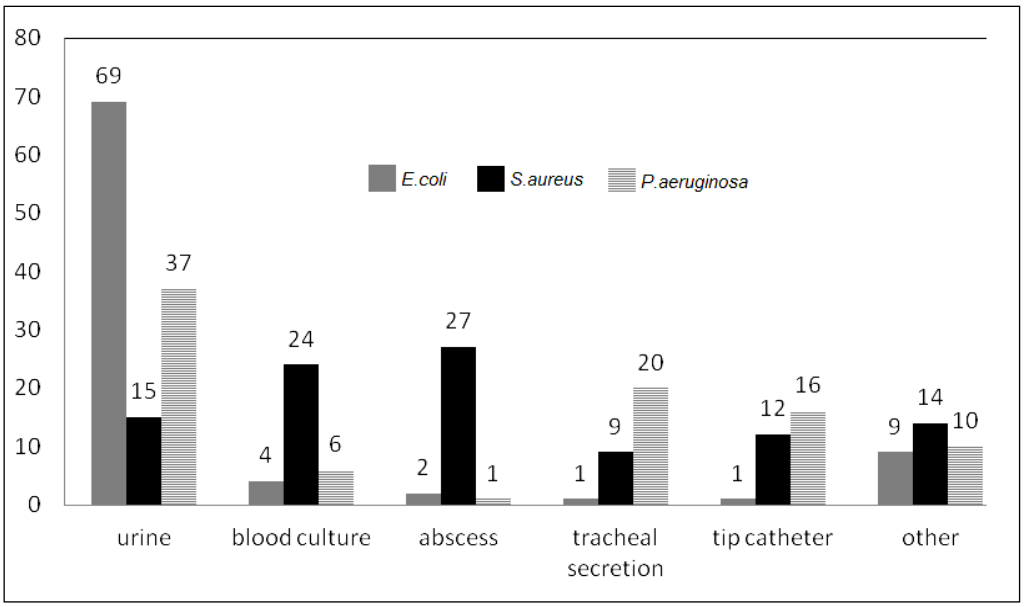


Figure 1 – Frequency of microorganisms by isolation source.

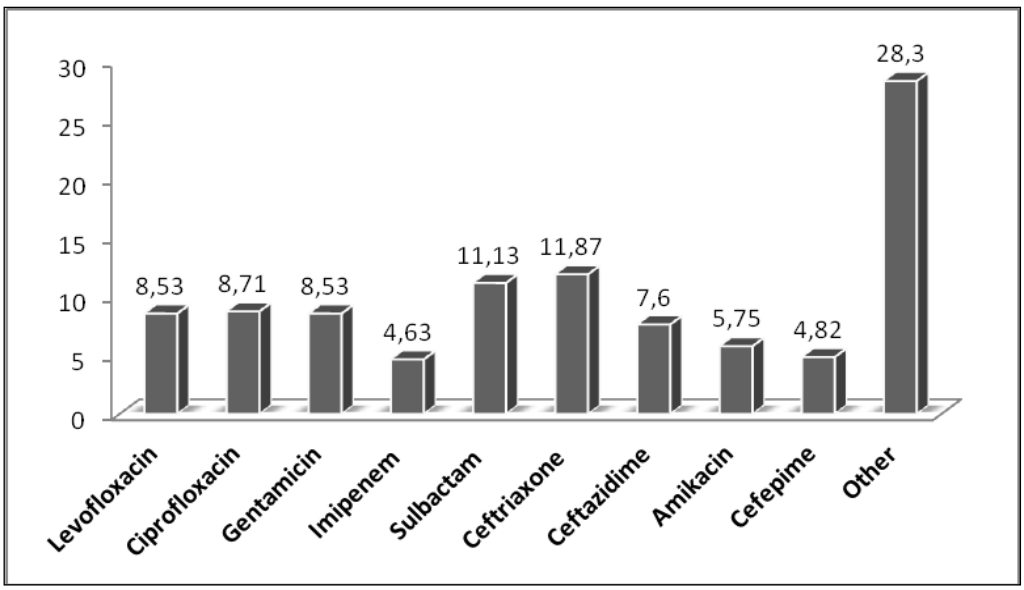


Figure 2a – Antibiotic resistance profile of *P.aeruginosa* strains

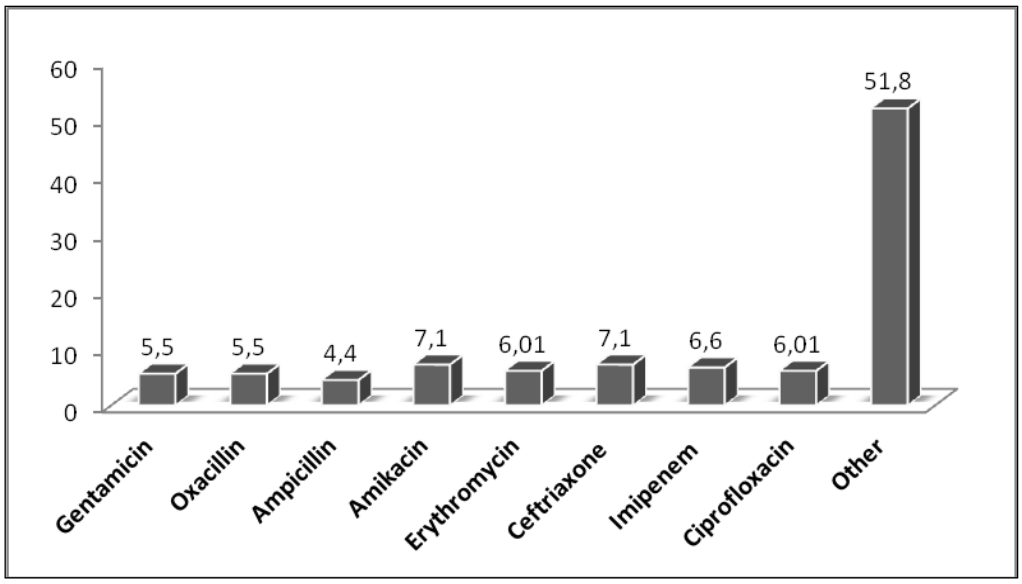


Figure 2b – Antibiotic resistance profile of *S. aureus* strains

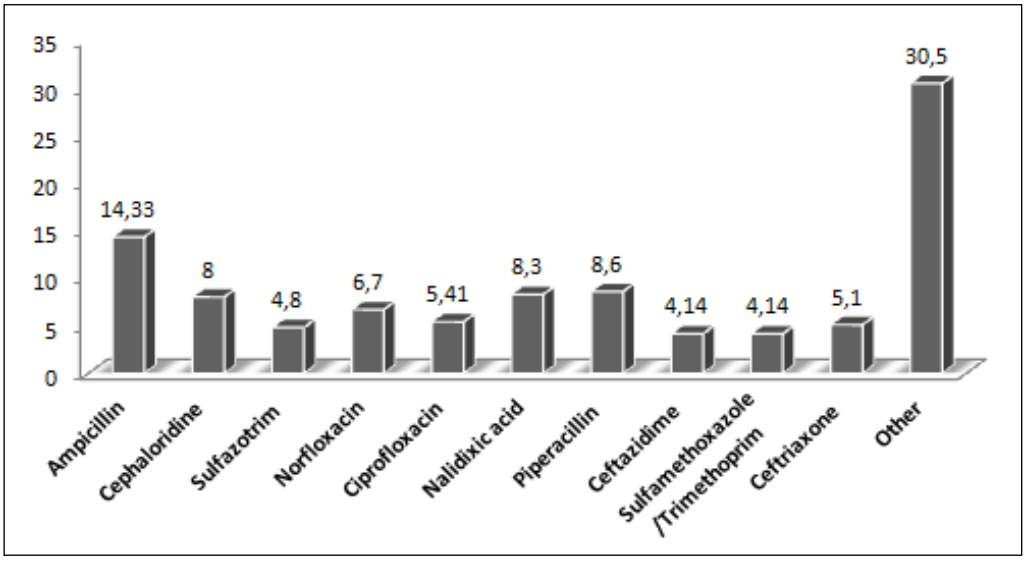


Figure 2c – Antibiotic resistance profile of *E. coli* strains

## Discussion

In the present study, the antimicrobial resistance profile of two hospitals from a poorly explored Brazilian region is described. Our data has some overlapping features with other studies developed in Brazil, in relation to the detection of hospital infections pathogens [12,13,14,18]. In the hospitals of this research, the rate of methicillin resistant *Staphylococcus aureus* (MRSA) and oxacillin resistant *Staphylococcus aureus* (ORSA) was considerably high, a probable result of an ineffective hygiene measures among professionals [14]. Damaged cutaneous barrier of patients can contribute to the development of infections. The mechanism of resistance to methicillin in *S. aureus* is mostly owed to the production of the modified penicillin-binding protein 2a, encoded by the *mecA* gene. High values were earlier described in African countries [9,10].

Infections caused by Gram-negative bacteria are of great concern worldwide. *E. coli* is the most common etiologic agent of Gram-negative infections, which are routinely treated with quinolones and fluoroquinolones, frequently prescribed antimicrobial drugs [3,4]. *Pseudomonas aeruginosa* is an opportunistic pathogen that affects immunocompromised individuals and causes life-threatening infections[2]. In this study, *E. coli* and *P. aeruginosa* were the most frequently isolated strains in urine samples, although they have been extensively described as biofilm producers in medical devices such as catheters and stents. Previous studies provided evidence of adequate sensibility to ciprofloxacin to varied species detected in nosocomial infections, including the tested ones in this research[2-4]. Interestingly, differing from such data, all strains of this study were resistant to ciprofloxacin.

*S. aureus* is the most frequent cause of skin and soft tissue infections, most of them acquired in hospital settings. *S. aureus* infections frequently begin as minor boils or abscesses and may progress to severe invasive infections that may disseminate to the lungs, heart or bones. Early infections are usually treated by incising and draining the lesion, combined to β-lactam drugs [16,17]. However, the resistance to such drugs among staphylococci is

steadily increasing, and the options of safe and enough active antimicrobial drugs are very scarce nowadays [18].

Diagnosis of bacterial infections is crucial for human health. The main focus of bacterial diagnostics has been to provide the adequate drug therapy, avoiding bacterial resistance and reducing possible side effects due to excessive exposion to antimicrobials.

## Conclusion

The increased microbial drug resistance has serious implications for clinical treatments. The varied pharmacodynamics of synthetic drugs might be investigated for the potential of chemical combinations for network polypharmacology; however, there are concerns that synergistic side effects would make selective combinations a danger.

Our findings are consistent with other reports, but this is the first prospective study in these hospitals to provide detailed epidemiological characteristics and outcomes of patients with varied infections. The impact of inappropriate antimicrobial therapy and resistant strains are very important and should, therefore, be verified by further molecular studies.

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