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Ciprofloxacin Sensitivity of *Staphylococcus* Strains Isolated at the Sylvanus Olympio University Hospital, Togo

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Abstract

Background: Staphylococcal infections are managed by hygiene measures and usage of antibiotics. The first-line treatment refers to beta-lactamins. However, the emergence of beta-lactamin resistant staphylococcal strains has been reported, as demonstrated by a study conducted in Lomé University Teaching Hospital, Bacteriology Laboratory (2001) on 742 *Staphylococcus aureus* strains which revealed 67.00% of them where methicillin resistant. In this setting of emergent methicillin-resistant strains, the second-line treatments are prescribed by clinicians without antibiograms. Fluoroquinolones are the first preference molecules used for second-line treatment because of their efficacy and affordability. We want to contribute to setting monitoring and alert-making tools for drug prescribers. Thus we conducted this study, aiming to determine the frequency of *S. aureus* and coagulase-negative staphylococci (CNS) strains isolation in different types of biologic samples, and to investigate the link between methicillin resistance and ciprofloxacin resistance. Methods: We conducted this study from January 2006 through Jun 2010. The Microbiology Laboratory Service collected and analyzed samples for diagnostic purpose from inpatients and outpatients consulted in the hospital. We collected and analyzed de-identified data on these patients to form laboratory records. Bacteriological analyses in which ciprofloxacin have not been

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tested were excluded. Results: Over the 1108 staphylococcal strains isolated from various biological samples processed, 751 were *Staphylococcus aureus* and 357 were coagulase-negative staphylococci. The strains sensitivity profile is for all isolates. The majority of germs were *S. aureus* and 20% of them were ciprofloxacin-resistant. The probability of a patient who has an infection caused by *S. aureus* increases his stay in high-risk settings such as intensive care unit, surgical intervention, extended hospitalization, use of a catheter. The use of broad-spectrum antibiotics increases the risk of multidrug-resistant strains. Conclusion: This study highlights the recurring issue of over consumption of antibiotics in nowadays medical treatments. There is a need to raise awareness about the rational use of antibiotics in general and fluoroquinolones particularly.

Keywords

***Staphylococcus*, Ciprofloxacin, Methicillin, Resistance**

1. Introduction

Staphylococci are ubiquitous germs part of the human and animal cutaneous-mucous flora. They can be spread by a healthy carrier. In all likelihood, they are secondary disseminated in the environment by squama and remain persistent because of their resistance to desiccation temperature variations and osmotic chock. When cutaneous-mucous barriers are damaged or when the host's defenses are diminished, theses germs induce cutaneous, bone, and visceral infections, even worse, they can lead to septicemia [1].

When hygiene measures are not respected and patients are note isolated in hospitals, staphylococcus infections can lead to hospital epidemics. Staphylococcus can also persist endemically and lead to a high rate of nosocomial infections. The seriousness of these infections is linked to symptoms severity and to the therapeutic difficulties because of the multi resistance to antibiotics [1] [2].

The management of infections requires hygiene measures and use of antibiotics. Beta-lactamins are used as first-line treatment. However, the emergence of beta-lactamin resistant staphylococcal strains has been reported. A study conducted at the Sylvanus Olympio University Hospital (CHU-Tokoin, name of the hospital at the time of that study), Laboratory of Bacteriology (2001) on 742 *Staphylococcus aureus* strains revealed that 67.00% of them where methicillin resistant (Methi-R) [3].

In the setting of emerging methicillin-resistant strains, second-line treatments are prescribed. These treatments are prescribed in our health services by clinicians without antibiogram evidences. The first preference molecules used for this purpose are fluoroquinolones because of their efficacy (good diffusion in adipose tissue) and affordability [4]. We conducted this study to assess ciprofloxacin resistance (Cipro-R) of staphylococcal isolates in our settings.

2. Materials and Method

Study design: We conducted a cross sectional study covering the period of January 2006 through June 2010. We collected disidentified data from the CHU-SO (precedently named CHU Tokoin), Bacteriology Laboratory records. These records are results of samples (vaginal swab, urethral swab, coproculture, urine, hemoculture, pus and antibiograms) from inpatients and outpatients consulted in the hospital, and were processed in the laboratory for diagnostic purpose only. Results of bacteriological tests for which ciprofloxacin was not tested were excluded.

Laboratory method: Methicillin-resistance and phenotypes of staphylococci antibiotics resistance is analyzed with agar diffusion method. The Mueller-Hinton agar is used for the antibiogram. Each *S. aureus* or CNS colony isolated is seeded in 4 ml sterile peptone-water contained in hemolysis tube. After 24 hours at 37˚C incubation in darkness, 2 or 3 drops of the broth media is added with a Pasteur pipette to 10 mL sterile distilled water contained in a tube with screw cap. The inoculum prepared is diluted until obtaining a turbidity visually equivalent to 0.5 McFarland [5].

Petri dishes are dried 15 min in a sterilizer. Dried dishes are seeded by inoculation, and the inoculum surplus is sucked up from the surface of the agar medium with a Pasteur pipette carried with a pear. A disk of each anti-

biotic is dispensed with a sterile plier: penicillin (P), kanamycin (K), tobramycin (T), gentamycin (G), lincomycin (L), erythromycin (E), pristinamycin (PT), ofloxacin (OFX), pefloxacin (PEF) or ciprofloxacin (CIP), vancomycin (V), oxacillin (OX) and cefoxitin (FOX). Oxacillin and cefoxitin are used to determine methicillin-resistance [6]. Oxacillin discs are tested on Muller-Hinton media added with 5% NaCl and dropped alone at the middle of the Petri plastic dish measuring 50 mm diameter. The other disks are dispensed by maximum of six in a Petri plastic dish measuring 90 mm diameter. The erythromycin disk is carefully dropped at 2 cm of the lincomycin disk. A pre-diffusion of antibiotics is carried out for 15 min at laboratory room temperature before incubation of dishes in a dark sterilizer at 37˚C for 18 to 24 hours without repetition.

Interpretation of the antibiogram is carried out following the guidelines of The French Society of Microbiology. Inhibition area diameters are measured with a ruler at 0.5 mm approximation [7]. Ciprofloxacin disc charge was 5 µg. *Staphylococcus* spp strains Cipro-R were those with inhibition diameter < 22 mm. Oxacillin disc and cefoxitin disc charge was respectively 5 µg and 30 µg. *Staphylococcus* spp strains were classified Methicllin-resistant if inhibition diameter was <20 mm or <25 mm respectively for oxacillin and cefoxitin.

Data analysis: The data were collected and processed with Epi Info Version 6.04 and Microsoft® Excel 2007 software. We compared values (Chi-square test and relative risk) at 0.05 significance level.

3. Results

Over a total of 1108 staphylococcus strains isolated during the study period, 751 (68%) were *S. aureus* and 357 (32%) were coagulase-negative staphylococci (CNS).The majority (53%) of staphylococci strains were isolated from inpatients versus outpatients (43%). With regard to biological samples, almost all staphylococci strains (82% of *S. aureus* and 72.98% of CNS) were isolated from pus samples (**Table 1**).

85.98% of *Staphylococcus aureus* strains isolated were ciprofloxacin sensitive (S), and 69.55% of CNS strains were ciprofloxacin sensitive. During the same period, the prevalence of ciprofloxacin resistant staphylococci strains increased significantly: $p < 0.01$ (**Figure 1**). The overall methicillin-resistance is 37.38%.

The relative risk (RR) of ciprofloxacin-resistant staphylococci of being methicillin-resistant was 1.85 and it was statistically significant ($p < 0.01$). The RR of ciprofloxacin-resistant CNS of being methicillin-resistant was 1.86 and it was statistically significant ($p < 0.01$) (**Table 2**). The RR of methicillin-resistant staphylococci of being ciprofloxacin-resistant was 2.54 and it was statistically significant ($p < 0.01$). The RR of methicillin-resistant CNS of being ciprofloxacin-resistant was 2.87 and it was statistically significant ($p < 0.01$) (**Table 3**).

4. Discussions

Upon this study, we found that there are more staphylococci strains isolated from inpatients than from outpatients settings. Several studies show that methicillin-resistant *Staphylococcus aureus* (MRSA) are being important bacterial pathogens in hospital setting, but our result (37.38%) is much higher: 6.64% in the United States (2010), 5.97% in Canada (1999) and 0.8% in Japan (2003) [8]-[10]. This could be explained by the emergence of resistant strains and increasing hospital acquired infections resulting from lack of good care practices and infections control guidelines.

Isolates originated from various samples. Pus and miscellaneous punctures were the most represented, followed by urine and hemoculture or blood culture. *S. aureus* was the most isolated from pus and miscellaneous punctures (83.62%) as compared to CNS strains (72.98%). As opposed to pus samples and miscellaneous punctures, there were more CNS strains (11.42%) than *S. aureus* (4.26%) isolated from hemocultures. This could be related to probable samples contamination during blood collection. This distribution is similar to the findings of Akoua-Koffi *et al*. (2004) in Côte d'Ivoire (64.7% from pus versus 9.4% isolated urine and other samples) and could be linked to the pathogenicity of staphylococcus strains according to the colonization site [11].

Our findings demonstrated an increasing rate of ciprofloxacin resistance. This could be induced by antibiotherapy practices. For example, self-medication of the population in the treatment of unproved typhoid and paratyphoid fevers, in view of the drug affordability and the various generic-type packaging of the molecule.

This ciprofloxacin-resistance is correlated with an increased methicillin-resistance as fluoroquinolone-resistance staphylococci have a methicillin-resistance profile. We found that ciprofloxacin-resistant *S. aureus* are 1.85-fold at higher risk of being methicillin-resistant. Methicillin-resistance is also correlated with an increased ciprofloxacin-resistance. Methicillin-resistant *S. aureus* are 2.54-fold at higher risk of being ciprofloxacin-resistant, meaning that failure of the first line treatment increases the risk of the second line treatment failure.

Table 1. Distribution of staphylococci by type of biological samples.

Type of sample	*S. aureus* (%)	S. CN (%)	Total
Pus	628 (83, 62)	262 (73, 39)	890
Urine	49 (6, 52)	46 (12, 88)	95
Blood culture	32 (4, 26)	41 (11, 48)	73
Vaginal swabs	23 (3, 06)	2 (0, 56)	25
Sperm	17 (2, 26)	3 (0, 84)	20
Urethral scraping	2 (0, 26)	3 (0, 84)	5
Total	751 (100)	357 (100)	1108

Table 2. Methicillin antibiogram profile of staphylococci strains according to their ciprofloxacin antibiogram profile.

	Methi-R	Methi-S	RR	P-value
Cipro-R *S. aureus*	112	74	1.84	< 0.01
Cipro-S *S. aureus*	291	601		
Cipro-R CNS	73	26	1.86	< 0.01
Cipro-S CNS	98	149		

Table 3. Ciprofloxacin antibiogram profile of staphylococci strains according to their methicillin antibiogram profile.

	Cipro-R *S. aureus*	Cipro-S *S. aureus*	RR	P-value
Methi-R	112	291	2.54	< 0.01
Methi-S	74	601		
	Cipro-R CNS	Cipro-S CNS	RR	P-value
Methi-R	73	98	2.87	< 0.01
Methi-S	26	149		

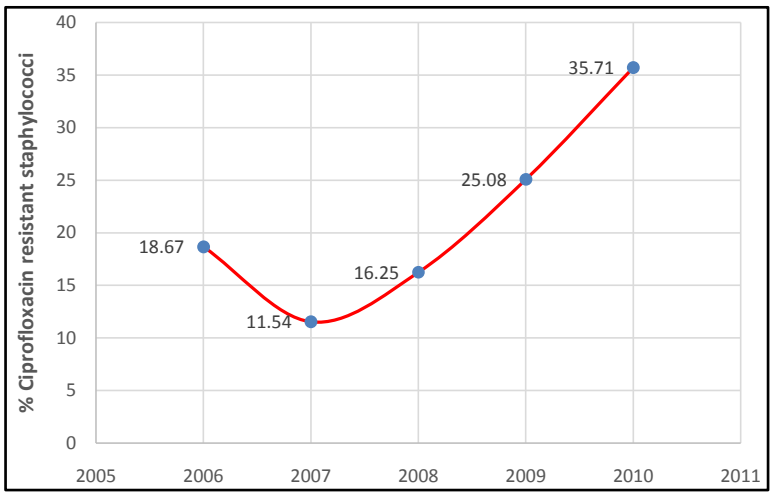

Figure 1. Prevalence and trends of ciprofloxacin resistant staphylococci strains.

5. Conclusion

This study highlights the recurring issue of over consumption of antibiotics in nowadays medical treatments. For better treatment outcomes, there is a need to raise awareness about the rational and well-targeted use of antibiotics in general and fluoroquinolones specifically. We recommend that, for patients displaying failure of the probabilistic in the first-line antibiotherapy (fluoroquinolones or betalactamins), the second-line treatment is considered on the basis of the antibiogram.

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