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Original article

Emergence of vancomycin-resistant *Staphylococcus aureus* identified in the Tlemcen university hospital (North-West Algeria)

Emergence de souches de Staphylococcus aureus *résistantes à la vancomycine isolées du centre hospitalo-universitaire de Tlemcen (Algérie Nord-ouest)*

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Abstract

Introduction. – Nosocomial infections are a matter of concern in surgical wards. Their incidence is constantly increasing, especially among immunocompromised patients who are vulnerable to colonization by opportunistic pathogens such as *Staphylococcus aureus*. The bacterium accumulates resistance mechanisms against antibiotics such as vancomycin. The objective of our study was to explore this resistance, to screen for *Staphylococcus aureus* strains resistant to vancomycin, and to try various antibiotic combinations against these strains.

Patients and methods. – The antibiotic susceptibility of 220 *S. aureus* strains was determined by agar diffusion and evaluation of minimal inhibitory concentrations (MICs), by dilution technique on solid medium according to clinical and laboratory standard institute (CLSI) standards. The screening of strains resistant to vancomycin was performed on brain heart infusion agar medium, supplemented with 6 μg/mL of vancomycin according to CLSI standards, and confirmed by determining MICs. The effectiveness of various antibiotic combinations was assessed by the checkerboard microplate method.

Results. – The results show multidrug resistance to agents known for their antistaphylococcal activity with fluctuations in the level of resistance.

Conclusion. – Three strains proved resistant to vancomycin. The vancomycin/gentamycin combination was the most effective.

Keywords: Antibiotic resistance; Antibiotic combination; *Staphylococcus aureus*; Vancomycin

Résumé

Introduction. – Les infections nosocomiales revêtent une allure préoccupante dans les services de chirurgie, leur incidence est en perpétuelle augmentation, surtout chez les immunodéprimés qui présentent une vulnérabilité à la colonisation par des germes pathogènes opportunistes tels que *Staphylococcus aureus.* Cette bactérie, cumule les mécanismes de résistance qui touchent actuellement des molécules telles que la vancomycine. L'objectif de notre travail a été d'explorer cette résistance, de rechercher des souches de *Staphylococcus aureus* résistantes à la vancomycine (SARV) et d'essayer différentes associations d'antibiotiques contre ces souches.

Patients et méthodes. – L'étude de la sensibilité aux antibiotiques de 220 souches de *S. aureus* a été déterminée par la technique de diffusion en milieu gélosé et l'évaluation des concentrations minimales inhibitrices (CMI) par la technique de dilution en milieu solide selon les normes du clinical and laboratory standard institute (CLSI). Le dépistage des souches résistantes à la vancomycine a été effectué sur milieu cœur cervelle additionné de 6 g/mL de vancomycine selon les normes CLSI et confirmé par la détermination des CMI. L'évaluation des différentes combinaisons d'antibiotiques a été réalisée par la méthode d'échiquier sur microplaque.

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Résultats. – Les résultats montrent une multirésistance touchant des molécules réputées pour leur action anti-staphylococcique avec des fluctuations dans le niveau de résistance.

Conclusion. – Trois souches se sont avérées résistantes à la vancomycine. L'association vancomycine-gentamycine s'est révélée la plus efficace.

Mots clés : Association d'antibiotiques ; Résistance bactérienne ; *Staphylococcus aureus* ; Vancomycine

1. Introduction

Hospital acquired infections are a matter of concern especially in high-risk units such as the surgical ward which manages patients who are extremely vulnerable to colonization and thus to infection [1]. *Staphylococcus aureus* is one of the most frequently incriminated bacteria in these infections.

Its pathogenicity, ubiquitous characteristic, and absence of nutritional requirements qualify this bacterium as an example of adaptation [2] and dissemination, especially when the skin barrier is ruptured. This adaptation also affects its aptitude to resist to several antibiotic agents, such as methicillin, which extends to resistance against most β-lactams and currently reach vancomycin considered until now as one of the last available antibiotic in this case [3]. These infections, which prevent the physician from treating the infecting bacteria, also increase the morbidity and mortality rates and induce an important over cost [4].

We studied the *Staphylococcus aureus* resistance of strains isolated in the Tlemcen teaching hospital (North-West of Algeria), and investigated new therapeutic alternatives by combining agents (bitherapy or tritherapy).

2. Patients and methods

Two hundred and twenty *Staphylococcus aureus* strains were isolated between April 16, 2007 and May 11, 2009, from 560 superficial samplings performed by swabbing postoperative surgical wounds of 287 patients presenting with nosocomial infection in the general surgery unit of the Tlemcen teaching hospital (658 beds).

The strains were identified by usual and biochemical tests allowing for discrimination among species: screening for catalase, staphylocoagulase, thermo-nuclease, and confirmation by the API Staph system (Bio Mérieux, France).

The antibiogram was performed by using the agar disk diffusion method according to clinical and laboratory standard institute (CLSI) standards [4].

The antibiotic disks used (Sanofi Diagnostic Pasteur, France): penicillin (10 UI), oxacillin (1 μg), streptomycin (30 μg), gentamycin (10 μg), tobramycin (10 μg), erythromycin (15 μg), fosfomycin (50 μg), clindamycin (2 μg), and vancomycin (30 μg).

Quality control was performed before every antibiogram on reference strains: *Staphylococcus aureus* ATCC 25923; *Pseudomonas aeruginosa* ATCC 27853; *Escherichia coli* ATCC 25922.

Screening for methicillin-resistant *Staphylococcus aureus* (MRSA) was performed on Mueller Hinton medium complemented by 4% of NaCl, and containing a final oxacillin concentration of 6 μg/mL according to CLSI standards.

Multiresistance was defined by resistance of the strain to at least three antibiotic agents [10].

Screening for vancomycin-resistant *Staphylococcus aureus* (VRSA) was performed on brain heart infusion agar (BHIA) containing a final vancomycin concentration of 6 μg/mL according to CLSI standards.

The minimal inhibitory concentration (MIC) of oxacillin, vancomycin, imipenem, gentamycin, cefotaxime, piperacillin, and rifampicin were determined by dilution method on solid medium [5].

The antibiotic combination effectiveness was assessed by the checkerboard microplate method [6], allowing quantifying of the interaction between two antibiotics: A and B. Various concentrations of the two antibiotics are used in the culture medium, imposing assessment of MICs for each of the two antibiotics and for the combination, while testing several couples of various concentrations.

Various combinations were tested: gentamycin + vancomycin, imipenem + gentamycin, piperacillin + vancomycin, imipenem + vancomycin, oxacillin + vancomycin, cefotaxime + vancomycin.

All these agents were injectable and were available at the Tlemcen teaching hospital.

3. Results

The patients having undergone surgery and presenting with pus discharge after 48 postoperative hours were included in this study. These patients were not given prophylactic preoperative antibiotic therapy. The mean age of patients was 48 ± 15 years, with 65% female and 35% male patients; the mean hospital stay was 9.25 days. Antri et al. (2010) reported that nosocomial MRSA infection was more important in adults over 50 years of age [7]. Likewise, Cosgrove et al., in 2005 reported that the length of hospital stay was extended by up to 2.6 days for patients with MRSA infection [4].

3.1. Study of antibiotic susceptibility

In Table 1, we report the multiresistant characteristics of MRSA presenting with cross-resistance to lactams extending to other antibiotic families, thus reducing the number of available therapeutic agents. All MRSA isolated in this study were resistant to penicillin.

These strains were also resistant to aminoglycosides with resistance rates ranging from 61.8% for streptomycin to 30.3% for gentamycin. This phenomenon also affected erythromycin

Table 1
Percentage of *Staphylococcus aureus* antibiotic resistance.
Pourcentage de résistance aux antibiotiques de Staphylococcus aureus.

Antibiotics	MRSA (*n* 165) (%)	MSSA (*n* 55) (%)	*Staphylococcus aureus* (220) (%)
Oxacillin	100	0	75
Penicillin	100	87.27	96.81
Gentamycin	30.3	7.27	24.54
Tobramycin	34.54	9	28.18
Streptomycin	61.8	36.36	55.5
Erythromycin	55.75	14.55	45.45
Fosfomycin	6.66	3.63	5.90
Clindamycin	12.12	16.36	13.18
Vancomycin	1.8	0	1.8

MSSA: methicillin susceptible *Staphylococcus aureus*; MRSA: methicillin-resistant *Staphylococcus aureus*.

and clindamycin with 55.75% and 12.12% of resistance respectively. Fosfomycin was more active with susceptibility rates superior to 90%.

The ten multiresistant strains reacted differently according to agents tested expressing fluctuations in MICs.

Vancomycin was also active on seven strains often with weak MICs (0.25–1 μg/mL). Three strains featured a high level of resistance, tolerating 6 μg/mL of vancomycin on BHIA in the screening test. These strains, which could be the first isolated in Algeria, have different MICs (16 μg/mL, 64 μg/mL, and 128 μg/mL) (Table 2).

3.2. Antibiotic combinations

The results of various combinations tested on the VRSA strain (Sa 84) are that out of six different antibiotic combinations, two combinations had a synergistic effect: vancomycin added to imipenem, and vancomycin added to gentamycin; the latter was the most effective with the lowest fraction inhibitory concentration (FIC) index (0.31). All combinations combining a β-lactam with vancomycin expressed an antagonistic effect except for imipenem added to vancomycin. This lack of effectiveness also affected the combination of gentamycin with imipenem (Table 3).

Table 2
Minimal inhibitory concentration (μg/mL) of ten multiresistant methicillin-resistant *Staphylococcus aureus* strains.
Concentrations minimales inhibitrices (μg/mL) des dix souches Staphylococcus aureus *résistant à la méticilline multirésistantes.*

	Minimal inhibitory concentrations (μg/mL)						
Strains	VA	OXA	IPM	GM	CTX	PIP	RA
Sa 84	128	>256	32	32	>512	>512	0.25
Sa 115	64	>256	64	32	>512	>512	0.5
Sa 31	16	>256	32	32	>512	>512	0.5
Sa 4	0.25	128	64	64	64	64	1
Sa 5	0.5	128	128	32	64	64	1
Sa 6	1	64	64	32	128	128	0.25
Sa 7	0.5	256	32	32	64	256	0.5
Sa 8	1	256	8	128	512	256	1
S 9	1	128	64	32	512	256	0.5
Sa 10	0.5	>256	128	64	128	256	0.25

VA: vancomycin; OXA: oxacillin; IPM: imipenem; GN: gentamycin; CTX: cefotaxime; PIP: piperacillin; RA: rifampicin.

4. Discussion

In Africa, the prevalence of MRSA is changing. It was close to 36% in Benin in 2006 [8] before decreasing in 2008 with a rate of 14.5% [9], whereas in Algeria, the rate of MRSA has been constantly increasing with 4.5% in 2002 [10], 33.2% in 2004 [11], and 45% in 2006 [12].

The results of this study on MRSA prevalence in the Tlemcen hospital surgery unit (Nord-West of Algeria) give a rate of 52%. This percentage, even though inferior to what has been reported for the U S A and Senegal with respectively 70% and 72% [13,14], is close to the one reported in Egypt in 2004 [15] but remains significantly superior to those reported for the Ivory Coast, Morocco, and Tunisia with respectively 25%, 19.3%, and 15.3% [16–18].

Indeed, the data may change in time from one ward to the other [1].

In France, the national observatory on the epidemiology of bacterial resistance to antibiotics (ONERBA) recorded a rates of MRSA at 35% between 1998 and 2003 [19], this percentage has been constantly decreasing [20] thanks to preventive measures against nosocomial infections such as antibiotic stewardship, isolation of patients, and global use of hydro alcoholic solutions recommended by the European antimicrobial resistance surveillance system (EARSS) [21]. In Algerian hospitals, Amazian 2006 reported an 18.6% rate of compliance to hygiene rules lack of available devices for hand hygiene and insufficient knowledge of adequate hygiene practices, which could account for the important diffusion of MRSA [22]. It is mandatory to associate screening on admission with hygiene measures and isolation of patients to effectively reduce new cases of colonization and MRSA infections [23]. These measures should also be complemented by recommendations such as specific training on the rational use of antibiotic for physicians, stressing the need to inform hospitals when referring patients colonized by MRSA, and insisting on the essential importance of healthcare personnel hygiene [24].

Algerian hospitals have been confronted to a spectacular increase of MRSA infections [12]. In a study performed at the Algiers Mustapha Bacha hospital, Antri et al. (2010) reported the dissemination of a clone named ST80-MRSA-SCC *mec* IV that could be of community origin [7]. The same clone was isolated in the Oran hospital (West Algeria) [12] and in the Didouche Morad hospital (Algiers) [25]. The recent increase of MRSA infections strongly suggests a horizontal transmission among patients [12]. According to Ramdani et al. 2006, it may be possible that patients admitted to the Mustapha Bacha hospital were nasal carriers on admission, or even that propagation was due to the healthcare personnel. The presence of an identical clone in France in a patient of Algerian origin confirmed its international distribution. Furthermore, older hospital clones were isolated in only 16% of cases [11].

Table 3
Synergy and antagonism of various antibiotics combinations against a vancomycin-resistant strain (fraction of inhibitory concentration index and minimal inhibiting concentration [μg/mL]).
Synergie et antagonisme des différentes associations d'antibiotiques contre une souche résistante à la vancomycine (index fraction of inhibitory concentration *et concentrations minimales inhibitrices [μg/mL]).*

Associations	Minimal inhibiting concentrations (μg/mL)					
A + B	MIC of A(alone)	MIC of B (alone)	MIC of A combined to B	MIC of B combined to A	IndexFIC	Interaction
GN + VA	32	128	8	8	0.31	Synergy
IPM + VA	32	128	16	8	0.56	Synergy
OXA + VA	256	128	512	128	3	Antagonism
PIP + VA	64	128	128	128	3	Antagonism
CF + VA	256	128	128	128	2.5	Antagonism
IPM + GN	32	32	32	32	2	Antagonism

GN: gentamycin; VA: vancomycin; IPM: imipenem; OXA: oxacillin; PIP: piperacillin; CTX: cefotaxime; FIC: Fraction of inhibitory concentration.

According to Seigel et al. (2007) [26], multiresistant bacteria are defined as microorganisms resistant to one or several classes of antibiotics. Other authors say that there is no consensual definition for multiresistance, and qualify a bacterium as multiresistant when resistant to at least three antibiotic classes.

An infection is said to be nosocomial, when it is associated to healthcare or occurs after at least 48 hours in hospital [27].

An infection is said to be community acquired when it occurs in a patient who was not hospitalized or hospitalized for less than 48 hours when symptoms first appeared. An MRSA infection is said to be community acquired if it fulfills the four following conditions:

- the infection was diagnosed in a patient not hospitalized or hospitalized for less than 24 hours;
- the patient had no history of MRSA infection or colonization in the previous year;
- the patient had not been hospitalized, had not been admitted to a long-term hospital unit, had not undergone surgery, or had not been dialyzed;
- the patient was not carrying a catheter or any other transcutaneous medical device [28].

Our relatively high rates of MRSA could be explained on one hand by the accumulation of several resistance mechanisms in these strains due to their genetic flexibility which let them acquire several mechanisms [2], and on the other hand, by a selection pressure induced by an inadequate use of antibiotic [29].

The increase of MRSA infections in Algerian hospitals has promoted the integration of glycopeptides in the therapeutic regimens aiming at the elimination of these strains. These agents were preserved from this resistance phenomenon until recently. Decreased susceptibility to vancomycin was first discovered in Japan in 1997, and rapidly spread worldwide with cases reported in the U S A, in Europe, and in Africa [30–32]. This dissemination was accompanied by the evolution of terminology; thus strains previously qualified as susceptible according to the national committee for clinical laboratory standards (NCCLS) [33], were then qualified as of intermediate susceptibility (VISA) according to new *Comité d'antibiogramme de la société Française de microbiologie* (CASFM) [34] and CLSI standards [5]. This change logically affected other categories (intermediate, resistant). This was complicated by the parallel emergence of a sub-population expressing heterogeneous resistance to vancomycin responsible for a number of therapeutic failures [35]. In this case, a low proportion reacts differently and reaches a MIC two to eight times more important than the parent cell, a prerequisite stage before acquisition of homogeneous resistance to vancomycin (VISA) [2], thickening of the cell wall, and increase of murein peptidoglycane monomers associated to a decrease of peptidoglycan reticulation facilitating affinity trapping of vancomycin and thus preventing its link with D-Alanyl D-Alanine of the newly formed peptidoglycan [36]. To be active, glycopeptides must cross peptidoglycan and bind to their targets on the cytoplasmic membrane. But, the greater the number of *S. aureus* cells is in an infection site, the more vancomycin molecules will be sequestered, inducing a kind of solubilization of the antibiotic and thus, a decrease of its tissue concentration. To prevent this, hospital physicians remove abscesses surgically and drainage the pus [37]. The first *S. aureus* strain highly resistant to vancomycin (VRSA) was isolated at the catheter port in a patient of a Michigan hospital [38]. Weigel et al. (2003) demonstrated that a genetic transfer from an *Enterococcus feacalis* strain to a MRSA allowed the passage of Tn 1546 carrying genetic determinants such as *van A* [39].

Antibiotic combinations remain an excellent alternative therapeutic, when confronted to such a resistance phenomenon, by preventing the emergence of resistance and widening the spectrum of action, which may reach deep polymicrobial foci.

These combinations, with their synergistic and bactericidal effects, feature rapid action and reduced toxicity [40]. The pharmacokinetics and tissue concentration of glycopeptides suggest using them in combination, especially against MRSA [41].

Vancomycin is known for its slow bactericidal activity and its nephrotoxicity but it remains effective if used at an inhibitory ratio (serum level/MIC) at least equal to eight [42,43].

Results proved that a combination of vancomycin and gentamycin was the most active. Published results are variable. Some authors, such as Goldstein et al., reported that combination of vancomycin with aminoglycosides was not appropriate for the treatment of glycopeptide intermediate *S. aureus* (GISA), but that it prevents septic shock [44]. Other authors stress the

effectiveness of arbekacin combined with vancomycin [45], or amikacin with vancomycin [46].

Out of the four combinations of a β-lactam with vancomycin, only the activity of imipenem was increased with vancomycin thus, inducing a synergistic effect. Indeed, several authors have reported that this combination was strongly active against MRSA [47,48] and some even mentioned against GISA [49].

An *in vivo* study proved the contribution of combining cefpirome with vancomycin for the treatment of severe MRSA infections in the ICU. Compared to vancomycin alone, this combination features better bactericidal kinetics, a rapid decrease of C reactive protein (CRP) rates, and absence of selection of other multiresistant bacteria [50].

Contrary to what was reported by Climo et al. [49], all our other combinations of β-lactams (piperacillin, cefotaxime, and oxacillin) with vancomycin had an antagonistic effect. These results correlate to the ones reported by Goldstein et al. in (2003) [51].

Our results prove that the combination of imipenem and gentamycin also expressed an antagonistic interaction. Some authors have reported the synergy between gentamycin and ampicillin by the action of the latter on the bacterial wall facilitating the entry of gentamycin and ending up in a faster bactericidal activity [52]. Novales et al. (2006), reported the synergy against *Staphylococcus* strains, especially between β-lactams and aminosides (cephalotin with amikacin and dicloxacillin amikacin) [46]. The notions of synergy and antagonism may be considered differently by the microbiologist or the hospital physician. The former will define it in terms of MIC or minimal bactericidal concentration (MBC), with effects measured *in vitro*, the latter in terms of patient cure or clinical failure [52].

In vivo, the results of clinical trial differ.

Paul et al. (2004) in a series of 7,586 patients compared treatment with a β-lactam, alone or in combination with an aminoside; the frequency of clinical failure was less important with the monotherapy whereas renal toxicity was higher with the bitherapy [53]. But in another study, combining a β-lactam with a fluoroquinolone resulted in a decreased death rate [54].

Combining third generation cephalosporins with fosfomycin is known for its synergy against MRSA but only if the bacterium is susceptible to fosfomycin.

This synergy may be explained by the action of fosfomycin, which decreases the expression of PLP 2 and 4, but also, that of PLP 2' known to support resistance in MRSA. This decreased expression of PLP 2, 4 and 2' is accompanied by an improved expression of PLP 3, which makes β-lactams effective [55].

5. Conclusion

Our study proved the high prevalence of MRSA in the surgery unit of the Tlemcen teaching hospital (Algeria), expressing multiresistance against several agents ranging from β-lactams, to aminosides and even vancomycin. The levels of resistance were often elevated considering the recorded MICs. Thus, three strains were resistant to vancomycin. This seems to reflect an inadequate use of antibiotics and the absence of a well-defined national policy. Using antibiotic combination such as vancomycin and gentamycin could be effective against strains with decreased susceptibility to vancomycin.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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