Original paper

Increasing rate of daptomycin non-susceptible strains of *Staphylococcus aureus* in patients with atopic dermatitis

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Adv Dermatol Allergol 2017: XXXIV (6): 547–552
DOI: https://doi.org/10.5114/ada.2017.72460

Abstract

Introduction: Daptomycin is a cyclic lipopeptide that is bactericidal against *Staphylococcus aureus*, including methicillin-resistant *S. aureus* (MRSA), vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA) strains. Daptomycin exerts its antimicrobial effect by a calcium-dependent interaction with the cytoplasmic membrane resulting in depolarization, ion loss and rapid cell death. Unfortunately, loss of daptomycin susceptibility in *S. aureus* in the clinical setting has been noted.

Aim: To evaluate the susceptibility profile to daptomycin among *S. aureus* strains isloted from patients with atopic dermatitis (AD). Another point was to correlate the results obtained by broth microdilution method and Etest, which is commonly applied in clinical setting.

Material and methods: One hundred patients with the diagnosis of atopic dermatitis were microbiologically assessed for the carriage of *S. aureus*. Antimicrobial susceptibility tests were performed using broth-microdilution (BMD) and Etests for daptomycin.

Results: *Staphylococcus aureus* strains were isolated from the majority of our patients, either from the skin (73%) or the anterior nares (75%). Six of the 100 nasal swabs (6%) and 5 of the 100 skin swabs (5%) were positive for methicillin-resistant *Staphylococcus aureus* (MRSA). A total of 81 of 148 (54.7%) daptomycin non-susceptible isolates of *S. aureus* were identified by BMD. Only 19 of 81 were also classified as non-susceptible by Etest.

Conclusions: Clinicians and microbiologists should be aware of the possibility of the emergence of daptomycin non-susceptibility (or increase in minimal inhibitory concentration) during prolonged therapy and closely monitor the susceptibility of persisting isolates that might be recovered during therapy.

Key words: atopic dermatitis, broth-microdilution, daptomycin, *Staphylococcus aureus*, vancomycin.

Introduction

Atopic dermatitis (AD) is a chronic, pruritic skin disease mainly affecting children which follows a remitting and relapsing course. It occurs in 10% to 20% of children and 1% to 3% of adults. Patients with AD have a unique predisposition to be colonized or infected by a number of microbial organisms, mostly *Staphylococcus aureus*. Eighty percent to 100% of patients with AD present nasal or skin colonization by *S. aureus*, while the prevalence in healthy individuals is 5% to 30%. An exacerbation of AD can be associated with bacterial infection; staphylococcal infections are the most common. An attempt was made to prove that eradication of *S. aureus* significantly reduces the severity of the disease [1–3]. Therapeutic options for multidrug-resistant Gram-positive pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) are limited. Daptomycin (DAP) seemed to be a promising candidate for new classes of anti-infectives. Daptomycin is a calcium-dependent cyclic lipopeptide produced by *Streptomyces roseosporus*, which shows a potent bactericidal activity against most Gram-positive organisms including MRSA [4]. Daptomycin has a clinically relevant activity against a variety of van-

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Received: 7.06.2016, **accepted:** 4.10.2016.

comycin-resistant organisms including *S. aureus* [5]. The mechanism of daptomycin action is unique: the drug kills bacteria in a concentration-dependent manner by binding preferential membranes of Gram-positive bacteria. After insertion, rapid depolarization occurs which leads to death of the bacterial cells due to disruption of critical metabolic functions, such as protein, DNA, and RNA synthesis [6]. Owing to its unique to its unique mechanism of action, it has been generally assumed that daptomycin-resistant organisms are difficult to generate. Unfortunately, therapeutic failures, albeit relatively uncommon, have been reported [7, 8]. Such non-susceptibility may occur in the absence of prior daptomycin exposure.

Aim

The main purpose of this study was to determine the antimicrobial susceptibility to daptomycin of *S. aureus* strains among patients with atopic dermatitis. In the present study, we also evaluated the correlation of daptomycin minimal inhibitory concentration (MIC) obtained by the Etest technique and the broth microdilution (BMD) method.

Material and methods

Patients and bacterial strains

Patients were enrolled in our study at the time of their visits to the Outpatient Clinic and during hospitalization in the Department of Dermatology, Venereology and Allergology in Gdańsk from August 2014 to August 2015. There was no selection of patients by sex or by severity of lesions. Atopic dermatitis (AD) was diagnosed following the criteria of Hanifin and Rajka, which include pruritus, typical morphology and distribution of eczematous lesions, chronicity of the disease and personal or family history of atopy [9].

The study was approved by the local Research Ethics Board (approval number NKBBN/242-477/2014). Voluntary informed consent in writing was obtained from all participants. The exclusion criteria included chronic dermatological condition with compromised skin barrier (e.g. psoriasis), diagnosis of any other chronic condition that increases the risk of MRSA colonization, oral or intravenous antibiotic treatment in the previous 4 weeks, treatment with topical antibiotics in the past 2 weeks, treatment with systemic corticosteroids or immunosuppressive drugs in the past 4 weeks, history of hospitalization, surgery, dialysis or residence in a long-term facility in the past year, indwelling catheter or a percutaneous device at the time of enrollment. Skin and nasal swabs were collected from 100 patients with AD to investigate the presence of *S. aureus*. The definition of Community Acquired-MRSA (CA-MRSA) was coined by the Centers for Disease Control and Prevention (CDC) in 2000. It refers to MRSA infection in a person who has none of the following established risk factors: isolation of MRSA more than 48 h after hospital admission; history of hospitalization, surgery, dialysis or residence in a long-term care facility within one year of the MRSA culture date; the presence of an indwelling catheter or a percutaneous device at the time of culture; or previous isolation of MRSA.

Identification of *S. aureus* and MRSA strains

Preliminary identification and detection of *S. aureus* and MRSA strains was conducted on the ChromID MRSA/ ChromID *S. aureus* biplate (bioMérieux) for the simultaneous detection of *S. aureus* and MRSA.

Antimicrobial activity

All *S. aureus* strains were used to determine the MIC using the broth microdilution method in Mueller Hinton broth according to the Clinical and Laboratory Standards Institute (CLSI) recommendations [10]. Assays for daptomycin were performed with and without medium supplemented with Ca^{2+} (50 mg/l). Polypropylene 96-well plates with bacteria at initial inoculums of 0.5×10^5 CFU/ml were exposed to daptomycin ranging concentrations (0.0625–32 µg/ml). All plates were further incubated for 18 h at 37°C. Minimal inhibitory concentration was taken as the lowest concentration of the compound at which a visible growth of bacteria was not observed. The experiments were performed in triplicate. Daptomycin non-susceptible strains (MIC > 1 µg/ml) were selected for Etests (bioMérieux). Mueller-Hinton (BBL) agar plates were used for the Etest. All setup and reading procedures were based on the manufacturer's instructions [11].

Statistical analysis

All statistical calculations were performed using the statistical package StatSoft. Inc. (2014) Statistica (data analysis software system) version 12.0. www.statsoft.com and Excel spreadsheet. Quantitative variables were characterized by the arithmetic mean, standard deviation, median, minimum and maximum values (range) and 95% CI (confidence interval).

To check whether a variable quantitative came from a normally distributed population, the Shapiro-Wilk analysis was used. In contrast, to test the hypothesis of equal variances, Leven (Brown-Forsythe) test was used.

The significance of differences between two examined groups, Student's *t* test or Mann-Whitney *U* were used. The significance of differences between more than two groups were checked by an F test (ANOVA) or Kruskal-Wallis. In all the calculations, the level of significance was assumed at $p = 0.05$.

Table 1. Susceptibility profile to Daptomycin of tested *Staphylococcus aureus* strains isolated from patients with atopic dermatitis (AD). Minimal inhibitory concentration was determined using the broth-microdilution method (BMD) with and without Ca^{2+} (50 mg/) supplementation (148 strains). Daptomycin non-susceptible strains (MIC > 1 µg/ml) were selected for Etest confirmation (81 strains)

No. of isolates	MIC range [µg/ml]		
	BMD	BMD (Ca^{2+})	Etest
S. aureus isolated from patients with AD (148)	0.25–16	0.0625–4.0	n/a
S. aureus Daptomycin non-susceptible (81)	8.0–16	2.0–4.0	from 0.016 to 256 (256 above norm)

n/a – not applicable.

Table 2. MIC50 and MIC90 values to Daptomycin of tested *Staphylococcus aureus* strains isolated from patients with AD. Minimal inhibitory concentration was determined using the broth-microdilution method (BMD) with and without Ca^{2+} (50 mg/l) supplementation (148 strains). Daptomycin non-susceptible strains (MIC > 1 µg/ml) were selected for Etest confirmation (81 strains)

No. of isolates	MIC range [µg/ml]					
	BMD		BMD (Ca^{2+})		Etest	
	MIC50	MIC90	MIC50	MIC90	MIC50	MIC90
S. aureus isolated from patients with AD (148)	8.0	16	2.0	4.0	n/a	n/a
S. aureus Daptomycin non-susceptible (81)	8.0	16	2.0	4.0	0.25	16

n/a – not applicable.

Results

Patients and bacterial strains

A total of 200 specimens were collected from 100 patients during the study. AD patients consisted of 55% of males and 45% of females, age: 1 to 63 years, median: 22.3 ±15.6 years. *Staphylococcus aureus* was reported in 75 of 100 (75%) skin swabs and 73 of 100 (73%) nasal swabs. Six of the 100 nasal swabs (6%) and 5 of 100 skin swabs (5%) were positive for MRSA (54.5% Community Acquired-MRSA, 45.5% Hospital Acquired-MRSA).

Antimicrobial susceptibility

In the following study, none of *S. aureus* strains were characterized as resistant to vancomycin in both, BMD method and Etest. In case of daptomycin, MIC determination was followed by two different methods – a broth microdilution (BMD) method and a gradient diffusion strip method (Etest). BMD was conducted in reference to the Clinical and Laboratory Standards Institute recommendations. Interestingly, the influence of Ca^{2+} medium supplementation (50 mg/l) was also examined. The Etest method was conducted according to manufacturer's instructions. In the first step, a total of 81 of 148 (54.72%) non-daptomycin-susceptible strains of *S. aureus* were identified by BMD in patients with AD. Strains isolated from patients with AD, which were non-susceptible to daptomycin were selected to Etest. Minimal inhibitory concentration determined in Ca^{2+} supplemented medium was 2-fold dilution lower (97.50% of strains) than in standard Mueller-Hinton medium (Table 1). For Etest, only 19 of 81 (23.45%) non-susceptible strains were classified as resistant. Minimal inhibitory concentration values generated with this method tended to be lower. In majority, up to 3 and 4 dilutions but +7 and –7 variations were also noted. There was also a poor correlation between BMD and Etest (correlation coefficient, $r = 0.306$ for confidence interval 0.95). Only 14.81% of the MIC values were within 1 dilution for values obtained by BMD. Minimal inhibitory concentration 50 and MIC 90 values for both methods were diametrically different (Table 2). For BMD it was 2.0 µg/ml and 4.0 µg/ml, respectively, while for Etest it was 0.25 µg/ml and 16 µg/ml. Since there is no intermediate interpretive category for daptomycin, only very major or major category interpretive errors can occur. In contrast to the BMD method, there were 76.54% of major errors (false susceptibility) determined by Etest.

Discussion

Epidemiology of *Staphyloccocus aureus* and MRSA strains in atopic dermatitis

Staphylococcus aureus strains were isolated from the majority of our patients, either from the skin (75%) or the anterior nares (73%). These results suggest that

the nose may act as a reservoir of *S. aureus* transferred from the skin surface by autotransmission. In 1961, first MRSA strains were reported. In 1980, MRSA strains became an endemic problem at different proportions at hospitals in several countries. Traditionally, infections caused by MRSA were limited to hospitals (Hospital Acquired-MRSA, HA-MRSA). Community-acquired infections (Community Acquired-MRSA, CA-MRSA) have been increasingly recorded since the last decade. The first report on CA-MRSA infection in a patient without any contact with the hospital environment was recorded in 1980 in the United States. There are few epidemiological reports on the colonization of methicillin-resistant *S. aureus* in atopic dermatitis. Worldwide studies suggest that the prevalence of MRSA in the population with AD varies from 0 to 30.8% [12]. In the USA, where CA-MRSA is now the most common pathogen cultured from patients with skin and soft-tissue infections in emergency departments, the colonization rate of AD patients is as high as 18.3% [13]. In the presented study, 6 of the 100 nasal swabs (6%) and 5 of 100 skin swabs (5%) were positive for MRSA (54.5% C-MRSA, 45.5% H-MRSA).

Daptomycin resistance

Daptomycin (DAP) is the first member of the antimicrobial agents approved for clinical use. Advantageous characteristics include a long serum half-life allowing once-daily dosing as well as antimicrobial activity against bacteria with reduced glycopeptide susceptibility [5].

Daptomycin is nowadays considered to be an antibiotic of choice for the treatment of biofilm-related infections. Daptomycin has been investigated with *in vitro* biofilm models [14, 15]. The rapid effectiveness is supported further by the observation that daptomycin eradicated MRSA in a biofilm after 3 days of 4-h daily exposures [16]. It was shown to act faster than minocycline, tigecycline, linezolid, vancomycin, and rifampin against *in vitro* central venous catheter biofilm infections [16].

The range of potential adaptations that may be associated with staphylococcal DAP resistance includes increased positive surface charge ('charge-repulsion hypothesis'), altered cell membrane fatty acid composition resulting in altered fluidity ('membrane order hypothesis'), enhanced cell membrane content of positively-charged phospholipids, as well as increased D-alanylation of the cell wall teichoic acid, resulting in reduced affinity of DAP to the cell membrane target, reduced permeabilization capacities, and reduced depolarization [17]. Mutations in mprF (a gene which contributes to membrane charge through lysinylation of PG), yycG (a histidine kinase gene of multiple functions, including impacts on membrane fatty acid biosynthesis), and rpoB and rpoC (subunits of RNA polymerase) have been found in *S. aureus* strains with daptomycin MIC greater than the susceptible range [18].

Several reports have linked increases in vancomycin MIC to increases in daptomycin MIC although no definitive mechanism has been elucidated. It may include increased cell wall thickness or reduced autolysis phenotypes, as commonly exhibited by strains with reduced susceptibility to vancomycin [19, 20]. However, such an association has not been seen in the presented study. The observation mentioned above suggests that frequent practice of using daptomycin when vancomycin therapy appears to be failing may be the wrong strategy.

Recent data have shown "cross-resistance" between DAP and cationic host defense peptides (HDPs) from neutrophils and platelets in *S. aureus* strains obtained from patients failing DAP therapy. Similar to many endogenous HDPs, daptomycin contains a significant peptide moiety that can be positively charged by calcium decoration during *in vivo* use. Therefore, one potential driver of such HDP-daptomycin cross-resistance phenotypes may be the capacity of innate HDPs to impact organisms before daptomycin therapy, facilitating increased daptomycin MICs on subsequent daptomycin exposure [21, 22]. Since many of the strains in the presented study were isolated from skin infection, it is quite possible to exhibit the daptomycin non-susceptibility during therapy because daptomycin exhibits cross resistance to other cationic host defense peptides.

Susceptibility testing

Susceptibility breakpoints for daptomycin are currently ≤ 1 µg /ml for methicillin-susceptible *S. aureus* (MSSA) and MRSA strains. Criteria for intermediate susceptibility or resistance have not been established because of lack of such strains. Organisms with a daptomycin MIC of > 1 µg/ml are considered non-susceptible. The Clinical and Laboratory Standards Institute (CLSI) recommends confirmation of non-susceptible strains by another method [11]. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has set both susceptible (MIC ≤ 1 mg/ml) and resistant (> 1 mg/ml) breakpoints for *Staphylococcus*. Disk diffusion testing is not recommended for daptomycin by the CLSI, EUCAST, or FDA until the method can be adjusted for appropriate detection of daptomycin-nonsusceptible strains. In contrast, Etest is frequently used by clinical laboratories to determine daptomycin MIC. The presented study confirms the previous reports that the concentration-dependent bactericidal activity of daptomycin requires physiological levels of free calcium ions (50 mg/ml) [23]. In the following study MIC determined in Ca^{2+} supplemented medium was 2-fold dilution lower (97.50% strains) than in standard Mueller-Hinton medium. Studies with daptomycin Etest by Fuchs *et al.* documented the pronounced effect of various calcium concentrations on MIC results [24]. Studies comparing the MIC results between the revised Etest method and the results of broth microdilution testing staphylococci have been reported recently [25]. Previous studies demonstrating a poor correlation among different methods for determining suscep-

tibility against daptomycin have reported that the Etest shows higher MIC results than the BMD reference method [26]. The lack of an intermediate category means that any category errors between a test method and the reference susceptibility test method can be categorized as either major (false resistance) or VM (false susceptibility). Further studies revealed 13% to 100% very major errors (VME) among MRSA strains with daptomycin-non-susceptibility MIC by BMD [27]. This study clearly demonstrates that there is a poor correlation of MIC results among the different methods used for susceptibility testing of daptomycin. Clinicians should be aware of the difficulties associated with susceptibility testing of daptomycin and interpret the non-susceptible results with caution.

Conclusions

Staphylococcus aureus presents significant clinical challenges because of its rising prevalence of antimicrobial resistance. Methicillin-resistant *S. aureus* (MRSA) infections have become a general occurrence in hospitals, and the situation is worrying since the pathogen is resistant to many antibiotics, including daptomycin and vancomycin, which were considered as the last resort for treatment of MRSA infections. Unfortunately, therapeutic failures have been reported, which correspond well with our findings (54.7% of strains non-susceptible to daptomycin). The possible explanation of a high number of the above-mentioned strains may be cross-resistance between daptomycin and cationic host defense peptides from neutrophils and platelets.

Acknowledgments

This study was partially supported by a grant from the Polish Ministry of Science for Young Scientist (Project No. 01-0221/08/508).

Conflict of interest

The authors declare no conflict of interest.

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