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NON-SUSCEPTIBILITY TRENDS AMONG METHICILLIN-RESISTANT COAGULASE-NEGATIVE STAPHYLOCOCCI ISOLATED FROM BLOOD CULTURES

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Abstract - Coagulase-negative staphylococci are a significant cause of hospital-acquired bacteremias. There is an increase of infections induced by methicillin-resistant strains, with growing resistance to other antibiotics. The aim of the study was to analyze the resistance of methicillin-resistant coagulase-negative staphylococci isolated from hemocultures in a five-year period. The study was carried out in the microbiology laboratory of the Institute for Pulmonary Diseases of Vojvodina, from 2008 to 2013. Coagulase-negative staphylococci were isolated from 196 hemocultures. Susceptibility tests were performed using the disc diffusion method. Of 196 coagulase-negative staphylococci, 122 (62.2%) were resistant to methicillin, of which 112 (91.8%), 105 (86.1%), 103 (84.4%), 88 (72.1%) were resistant to erythromycin, gentamicin, ciprofloxacin and clindamycin, respectively. All strains were susceptible to vancomycin and linezolid. Multiple resistance was registered in 100 (82%) strains. The most common resistance pattern was gentamicin-erythromycin-clindamycin-ciprofloxacin. Multiple resistance was established in a significant percentage of methicillin-resistant strains.

Key words: Coagulase-negative staphylococci, methicillin-resistant, susceptibility, blood culture

INTRODUCTION

Coagulase-negative staphylococci (CoNS) represent a group of opportunistic microorganisms that are common inducing agents of bacteremias and other hospital infections, particularly in the patients with medical implants (central and peripheral venous catheters, valvular prostheses, artificial heart valves, pacemakers and orthopedic prostheses), as well as in immunocompromised subjects (Stojanović et al., 2008; Hidron et al., 2008). Although coagulase-negative staphylococci have for years been among the most common bacteria isolated from blood cultures both worldwide (Laupland et al., 2013) and in Serbia (Kulauzov et al., 2008; Šuljagić et al., 2006; Jovanović et al., 2003.). These organisms are skin commensals, and are considered the most common blood culture contaminants (Elzi et al., 2012.). The treatment of the patients with coagulase-negative staphylococcal strains isolated from hemocultures has been doubtful and diverse clinical guidelines have been proposed suggesting catheter removal with no antibiotic therapy applied (Mermel et al., 2009). However, serious complications have been registered due to the omitted antimicrobial treatment in patients with coagulase-negative staphylococci isolated from blood cultures, resulting in prolonged hospitalization, although mortality increase has not been confirmed. An adequate early antibiotic therapy may improve the final treatment outcome in the patients with CoNS isolated from hemocultures that play a particularly important

role in patients in whom the clinical symptoms of the infection persist more than 48 hours (Molina et al., 2013). A significant increase in infections induced by methicillin-resistant staphylococci with concurrent resistance to other antibiotics has been registered (Koksal, 2009). Methicillin resistance of CoNS is due to the expression of the penicillin-binding protein 2a (PBP2a), which is a transpeptidase with a low affinity for β-lactams, encoded by the mecA gene (Zong et al., 2011). The mobile genetic element, the staphylococcal cassette chromosome (SCCmec), is responsible for transmission of the mec genes determining the resistance to methicillin and almost all beta-lactam antibiotics (Ito et al., 2003). The horizontal transmission of the resistance genes from methicillin-resistant CoNS onto *Staphylococcus aureus* has been confirmed, contributing significantly to the development of methicillin-resistant *S. aureus* (Fluit et al., 2013). The resistance of CoNS to β-lactams has been increasing, as well as resistance to other antibiotics, including aminoglycosides, quinolones, macrolides and tetracyclines (Casey et al., 2007).

The objective of this study was to analyze the resistance of coagulase-negative staphylococci isolated from blood cultures in a five-year period.

MATERIALS AND METHODS

The retrospective study included 196 strains of coagulase-negative staphylococci isolated from blood cultures in the Center for Microbiology, Immunology and Virology of the Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica (Serbia), from January 2008 to January 2013. Blood samples were taken from patients hospitalized in the Institute for Pulmonary Diseases of Vojvodina and the Institute for Cardiovascular Diseases of Vojvodina. For blood sampling, 10 ml of blood was collected. Samples were immediately transferred to blood culture bottles for aerobic and anaerobic cultivation. Incubation was performed using the automated BacT/Alert system (BacT/Alert 3D, Biomerieux, France). Positive blood cultures were inoculated using a blood agar base supplemented with 5% sheep blood. After incubation at 37°C for 24h, macroscopic examination was performed. CoNS were detected based on colony morphology, Gram staining, the absence of coagulase activity and growth on Chapman salt agar. The final identification was performed using commercial identification systems for Gram-positive bacteria (BBL Crystal Identification Systems Gram-positive ID kit, Beckton Dickinskon, USA). For each CoNS strain, antimicrobial susceptibility was determined by the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI).

The statistical analysis of the results was performed using the Statistical Package for the Social Sciences (SPSS), version 20. Results were expressed through descriptive statistics, as simple frequencies and percentages. The chi-squared test was used for determination of statistically significant differences. Trends were analyzed by linear regression method. The tested significance level was $\alpha=0.05$.

RESULTS

The study included 196 blood cultures of indoor patients treated in the Institutes in Sremska Kamenica with isolated CoNS. The patients from whom the isolates were obtained included 114 (58.2%) males and 82 (41.8%) females. The patients' mean age was 68 (SD ±17) years. The samples were taken from 103 (52.6%) patients hospitalized in the Institute for Pulmonary Diseases of Vojvodina and from 93 (47.4%) indoor patients treated in the Institute for Cardiovascular Diseases of Vojvodina.

The distribution of isolated CoNS species is shown in Table 1. Fig. 1 shows the distribution of CoNS in respective years.

From isolated CoNS strains, 147 (75%) were resistant to erythromycin, 123 (62.8%) to ciprofloxacin, 122 (62.2%) to gentamicin and 112 (57.1%) to clindamycin. All CoNS isolates were susceptible to vancomycin and linezolid. Fig. 2 shows the overall antimicrobial susceptibility of CoNS isolates from blood samples in the 5-year period.

Table 1. Distribution of CoNS species.

Species	x	%
S. epidermidis	85	43.4
S. haemolyticus	66	33.7
S. hominis	19	9.7
S. capitis	10	5.1
S. warneri	9	4.6
S. simulans	3	1.5
S. saprophyticus	3	1.5
S. conhii	1	0.5
In total	196	100

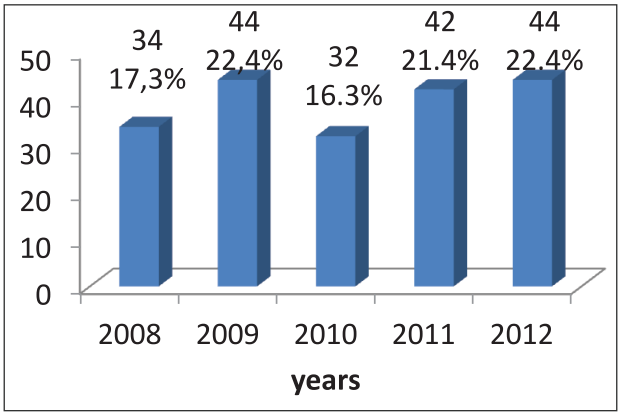

Fig. 1. Distribution of CoNS in respective years.

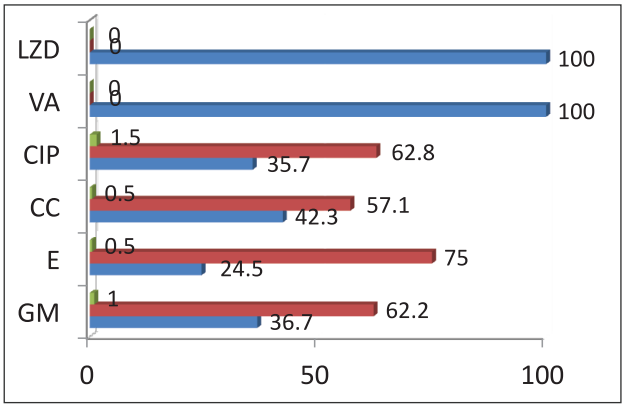

Fig. 2. Antimicrobial susceptibility of CoNS.

**LZD-linezolid, VA-vancomycin, GM-gentamicin, CIP-ciprofloxacin E-erythromycin, CC-clindamycin; I-intermediate sensitive, R-resistant, S-sensitive.*

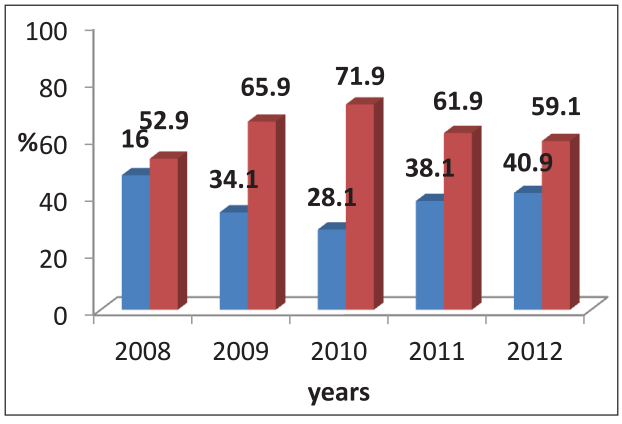

Fig. 3. Distribution of methicillin-resistant and methicillin susceptible CoNS.

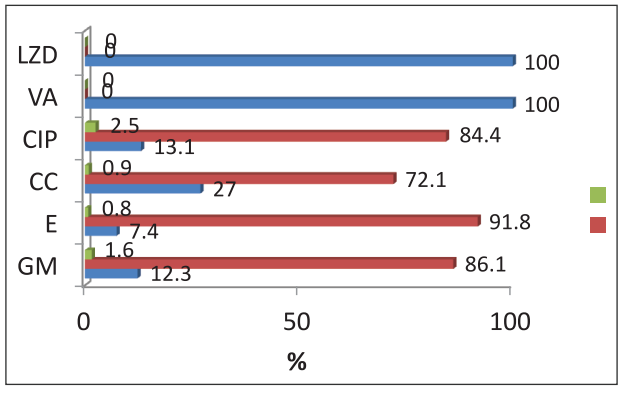

Fig. 4. Antimicrobial susceptibility of methicillin-resistant CoNS.

**R-resistant, S-sensitive*

An increasing non-susceptibility year-to-year trend is detected for ciprofloxacin (R=0.969, p=0.006). For other antibiotics, there were no smooth year-to-year trends, although some differences were noticed.

Fig. 3 shows the distribution of methicillin-susceptible and methicillin-resistant CoNS isolates in the respective years. No significant difference was registered in methicillin-resistant CoNS distribution in the studied 5-year period ($\chi 2 = 2.955$; $p = 0.565$).

Of 196 CoNS isolates, 122 (62.2%) were non-susceptible to methicillin; 112 (91.8%) bacterial strains non-susceptible to methicillin were non-susceptible

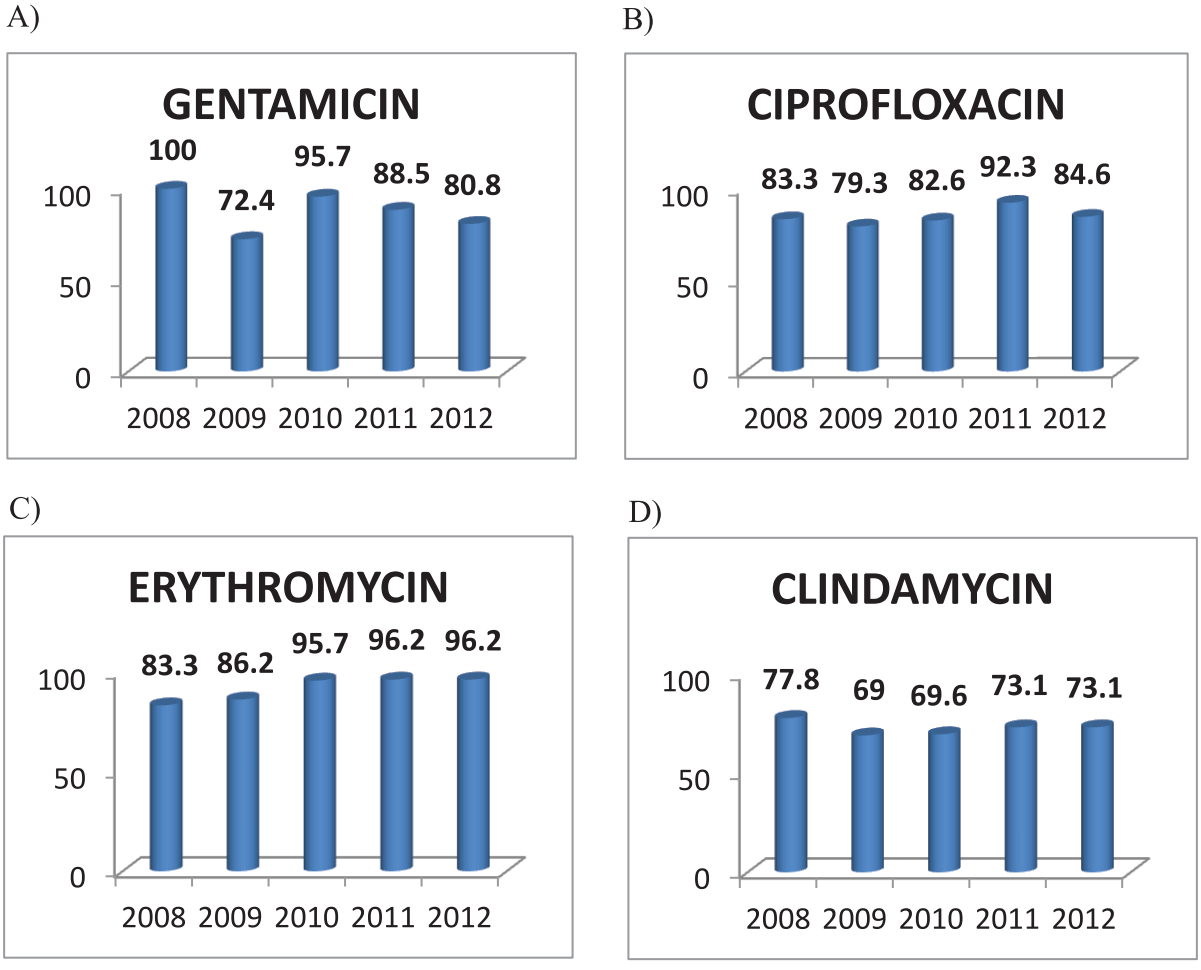

Fig. 5. Non-susceptibility of methicillin-resistant CoNS to gentamicin, ciprofloxacin, erythromycin, clindamycin in respective years.

**LZD-linezolid, VA-vancomycin, GM-gentamicin, CIP-ciprofloxacin E-erythromycin, CC-clindamycin; I-intermediate sensitive, R-resistant, S-sensitive.*

to erythromycin, 105 (86.1%) strains were non-susceptible to gentamicin, 103 (84.4%) strains to ciprofloxacin and 112 (72.1%) strains were clindamycin-resistant. No CoNS were found resistant to linezolid and vancomycin (Fig. 4).

Fig. 5 shows the non-susceptibility of methicillin-resistant CoNS to gentamicin (A), ciprofloxacin (B), erythromycin (C), and clindamycin (D) in the respective years. An increasing non-susceptibility year-to-year trend is detected for erythromycin (R=0.903, p=0.036). For other antibiotics, there were no smooth year-to-year trends.

Table 2 shows the resistance patterns among methicillin-resistant CoNS. Multiple resistance was registered in 100 (82%) methicillin-resistant CoNS strains. The most common resistance pattern was gentamicin-erythromycin-clindamycin-ciprofloxacin registered in 71 (58.2%) isolates.

DISCUSSION

Coagulase-negative staphylococci are ubiquitous commensals of the skin and mucous membranes. The importance of these bacteria as potential agents of infections, particularly hospital ones, has been increas-

Table 2. Resistance patterns of methicillin-resistant CoNS.

No of resistance markers	Resistance patterns of CoNS	No of isolates
0	/	1
1	GM	1
	CIP	1
	E	3
2	CC-CIP	1
	GM-E	2
	E-CIP	3
	E-CC	4
	GM-CIP	6
3	E-CC-CIP	4
	E-GM-CC	8
	E-GM-CIP	17
4	E-GM-CC-CIP	71
In total		122

**GM-gentamicin, CIP-ciprofloxacin E-erythromycin, CC-clindamycin.*

ing in recent years (Koksal et al., 2009). Although the CoNS strains isolated from blood cultures have been recognized as potential agents of bloodstream infections, it is not always easy to recognize their significance, particularly when only one blood culture is positive (Elzi et al., 2012). For years, CoNS have been regarded as a contamination due to inadequate blood sampling, sample transportation or laboratory examination (Šuljagić et al., 2006). However, the importance and role of these low virulence bacteria as bloodstream infection agents have been revealed in recent years (Casey et al., 2007; Molina et al., 2013). Although they rarely induce severe sepsis or septic shock, CoNS-induced blood infections are not without consequences for a patient. A delayed antimicrobial therapy may prolong the hospital treatment and contribute to increased mortality rates (Molina et al., 2013). As coagulase-negative staphylococci are quickly adaptable to antibiotic stress, an increasing use of broad-spectrum antibiotics has induced the growth in resistance (Casey et al., 2007). In order to apply the empirical antibiotic therapy, it is necessary to have an insight into the resistance patterns of the most common strains.

About 15 strains of coagulase-negative staphylococci are most frequently isolated from clinical samples, of which *S. epidermidis*, *S. haemolyticus*, *S. warneri*, *S. hominis*, and *S. saprophyticus* exhibit an elevated resistance to various antimicrobial drugs (Sheikh et al., 2012). In our five-year study, eight CoNS strains were identified from blood cultures. The most common CoNS strains were *S. epidermidis*, *S. haemolyticus* and *S. hominis*, while *S. saprophyticus* and *S. conhii* were the fewest. Stojanović et al. (2008) identified *S. epidermidis* as the most common CoNS strain in their study, and also found a fewer number of *S. haemolyticus*, *S. simulans*, *S. capitis*, and *S. saprophyticus*. Similar studies done by Koksal et al., Olivares et al., Sheikh et al., and Hope et al. found *S.*

epidermidis, *S. haemolyticus* and *S. hominis* to be the most common CoNS isolated from blood cultures. *S. capitis*, *S. warneri*, *S. simulans*, *S. saprophyticus* and *S. conhii* were more rarely isolated CoNS from blood cultures (Koksal et al., 2009; Olivares et al., 2011; Hope et al., 2008).

Of all CoNS isolated in our five-year study, 75% were resistant to erythromycin, 62.8% to ciprofloxacin, 62.2% to gentamicin, while clindamycin resistance was registered in 57.1% of isolated strains. All CoNS strains were susceptible to vancomycin and linezolid. Kulauzov et al. reported 68.7% CoNS strains isolated from blood cultures as erythromycin resistant, 57.4% were resistant to gentamicin, while 46.1% and 38.5% of isolates were resistant to ciprofloxacin and clindamycin, respectively. In the study performed by Jovanović et al. (2003), the most CoNS strains isolated from blood cultures were resistant to amikacin and ciprofloxacin (76.5% and 68.6%, respectively), while 52% and 47.5% of the strains were resistant to gentamicin and erythromycin, respectively. Such differences in resistance to particular antimicrobial drugs may be explained by differences in the use of antibiotics.

The administration of methicillin in the treatment of penicillin-resistant strains induced the development of methicillin-resistant strains that have become increasingly frequent agents of hospital-acquired bacteremia. The increasing resistance of CoNS has been documented by the fact that resistance to methicillin was 2% in the 1980s, reaching 80-90% in recent years (Olivares et al., 2011). In our study, 62.2% of CoNS isolated from blood cultures were resistant to methicillin. Methicillin resistance ranged from 52.9% to 71.9%. A positive resistance trend of methicillin-resistant CoNS was not registered in the examined five-year period. In Great Britain, 67% methicillin-resistant CoNS strains were isolated from blood cultures over the period from 1997 to 2002 (Casey et al., 2007). Koksal et al. reported 67.5% of methicillin-resistant CNS strains isolated from blood cultures in Turkey. In their five-year study, Hope et al. reported methicillin resistance of CoNS isolated from blood cultures ranging from 54.2% to 79.9%, correlating well with our results. Some authors report a very high (over 80-90%) methicillin resistance of CoNS strains isolated from different clinical samples (Olivares et al., 2011; Sheikh et al., 2012). Depending on the region, methicillin resistance in Europe ranges from 70% to 80%, and similar values are reported in the United States, Canada and South America (Sheikh et al., 2012; John et al, 2007).

Besides the increasing problem of methicillin resistance which eliminates all β-lactam antibiotics from the treatment of staphylococci-induced infections, an increasing resistance to other groups of antimicrobial drugs has been registered, including resistance to aminoglycosides, quinolones, macrolides and tetracyclines (Livermore, 2000; Casey et al., 2007). In our study, among methicillin-resistant strains, 91.8% were resistant to erythromycin, 86.1% to gentamicin, 84.4% to ciprofloxacin and 72.1% to clindamycin. All examined strains were sensitive to vancomycin and linezolid. Our results are in agreement with those reported by Santos Sanches et al., who established resistance to erythromycin, ciprofloxacin, clindamycin and gentamicin as the most frequent in Spain. In Turkey, Koksal et al. also reported a very high resistance to gentamicin (90%), erythromycin (80%), clindamycin (72%) and ciprofloxacin (67%). Hope et al. reported the highest resistance to erythromycin (80.2%) in Great Britain, followed by high resistance to gentamicin (73.4%) and ciprofloxacin (67.1%), while resistance to clindamycin was significantly lower (25.5%). The authors reported great differences in the sensitivity of methicillin-susceptible and methicillin-resistant CoNS In Europe, resistance to gentamicin ranges from 50.2 to 61.3%, while it is somewhat lower in the United States, amounting to 32.4% (John et al., 2007).

Analyzing all coagulase-negative staphylococci in our five-year study, a positive resistance trend has been registered for ciprofloxacin. The analysis of methicillin-resistant strains has only revealed a positive resistance trend for erythromycin. In their five-year study, Hope et al. did not register a positive resistance trend for any of the examined antibiotics

although some evidence for year-to-year fluctuations was detected. A positive resistance trend has been noticed for gentamicin, ciprofloxacin, clindamycin, and erythromycin in the United States and worldwide in the large-scale, multicentric study SENTRY (Diekema et al., 2001).

Methicillin-resistant CoNS often exhibit a concurrent resistance to three or more antimicrobial drugs. In our study, multiple resistance has been registered in 81.9% of methicillin-resistant strains. The most common resistance type is erythromycin-gentamicin-clindamycin-ciprofloxacin, established in 58.2% of multiresistant strains. The hospital CoNS strains are more resistant than community ones, and often exhibit multiple resistance (Hope et al., 2008; John et al., 2007). Among CoNS, multiresistance is most frequently found in *S. haemolyticus*, averaging 2.8 (John et al., 2007). Hope et al. reported 84% of *S. haemolyticus* and 70% of *S. epidermis* were resistant to three or more antibiotics, including ciprofloxacin, erythromycin, gentamicin, oxacillin, teicoplanin and tetracycline. Monsen et al. have confirmed the presence, survival and spread of the clones of multiresistant strains of methicillin-resistant CoNS in a hospital environment. Multiresistant strains are most often registered in hospital units where antibiotics are excessively applied (Monsen et al., 2005). A reduced use of antibiotics would prevent multiresistant CoNS strains developing.

Multiresistant CoNS represent a major issue as these infections can be treated by vancomycin and linezolid only. The CoNS strains examined in our study were 100% sensitive to vancomycin, the drug of choice for the treatment of CoNS-induced infections. These results correlate positively to those reported by Olivares et al. Besides vancomycin, 100% sensitivity to linezolid was also established in our study. However, a wide and continuous use of linezolid has induced resistance to this drug as well (Olivares et al., 2011). In long-term-treated patients, it is suggested to change antibiotics in order to avoid a permanent selective pressure and the development of resistance.

Multiresistant CoNS strains may colonize the hospital environment and thus become a source of antimicrobial resistance. The treatment of infections induced by these strains may become a great challenge, as the therapeutic capacities of the existing antibiotics have been rapidly exhausted. It is therefore crucial to undertake preventive measures, including the rational use of antibiotics and to prevent multiresistant CoNS from spreading.

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