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Research article

Distribution of *mecA* gene amongst *Staphylococcus aureus* isolates from Southwestern Nigeria

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ABSTRACT: Staphylococcus aureus (S. aureus) is an important pathogen in human infections and is implicated in a wide variety of infections, from mild skin infections to more serious and invasive infections. Methicillin resistant S. aureus (MRSA) is one of the major causes of nosocomial infection in hospital setting. The study was aimed at determining the distribution of mecA gene among the S. aureus isolates from south western tertiary hospitals, Nigeria. In this study, a total of 194 isolates of S. aureus were collected from the specimen submitted to diagnostic section of Medical Microbiology Department, University College Hospital (UCH) Ibadan, Obafemi Awolowo University Teaching Hospital, Ile-Ife, and LAUTECH Teaching Hospital (LTH) Osogbo, in the South Western Nigeria. Antibiotics susceptibility testing including methicillin sensitivity testing, beta lactamase testing, PCR for detection of mecA gene, and minimum inhibitory concentrations to methicillin were carried out on all the 194 isolates of S. aureus. Among the 194 strains, 40 (20.6%) were MRSA using 10 µg methicillin disc. PCR analysis showed that mecA gene was present only in 43 (22.2%) of 194 S. aureus isolates. Minimum inhibitory concentration (MIC) was also carried out to determine the degree of resistant of the isolates showed that the MIC₅₀ and MIC₉₀ for MRSA or mecA⁺ were > 128 μ g/ml while the MIC₅₀ and MIC₉₀ for mecA were 0.5 and 2 μ g/ml, respectively. Furthermore, 124 (64 %) of the 194 S. aureus isolates were β-lactamase producers. The study found that there were strong associations between isolation site of specimens / nature of specimens ($X^2 = 16.74$; p < 0.05), beta lactamase producing S. aureus strains ($X^2 = 29.21$; p < 0.05) and no association was found between the hospitals, gender, age and the prevalence of MRSA in this study (p > 0.05). The study concludes that the prevalence of mecA gene in S. aureus in South Western, Nigeria is 22% and mecA gene detection is a good predictor of methicillin resistance in S. aureus in Nigeria, hence use as a method of detection of MRSA

Keywords: Staphylococcus aureus, mecA gene, MRSA, antibiotic resistance, Nigeria.

INTRODUCTION

Staphylococcus aureus is an important pathogen in hospital and community settings. Methicillin resistant Staphylococcus aureus (MRSA) was first isolated soon after introduction of methicillin into clinical use in 1960 (Barber, 1961; Jevons, 1961). MRSA is a strain of S. aureus that is resistant to methicillin or beta lactamase resistant group of penicillin and usually these strains of S. aureus are usually resistant to more than

one antibiotic and because of this, the infections due to this strain of MRSA are very difficult to treat. The pathogenicity of *S. aureus* infections is associated with various bacterial surface components (e.g., capsular polysaccharide and protein A), including those recognizing adhesive matrix molecules (e.g., clumping factor and fibronectin binding protein), and to extracellular proteins (e.g., coagulase, hemolysins, enterotoxins, toxic-shock syndrome (TSS) toxin, exfoliatins, and Panton-Valentine leukocidin (PVL) (Archer, 1998), and MRSA strains being group of *S. aureus* are likely to have one or more of these pathogenicity traits.

The incidence of MRSA has been on the increase in the world since it first reported. Subsequently, the occurrence of MRSA particularly in hospitalized patients has increased steadily and nosocomial infections caused by such strains have become a serious problem worldwide. Methicillin resistant strains of *S. aureus* produce a new penicillin binding protein (PBP),

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otherwise known as PBP2' or PBP2a (Hartman and Tomasz, 1984, Utsui and Yokota, 1985), with a low affinity for β -lactam antibiotics. This protein is encoded by mecA gene (Ubukata et~al., 1985).

In the clinical microbiology laboratory, MRSA can be detected using disc diffusion and broth and agar dilution tests. The detection of MRSA is not a clear cut test when compare to other antibiotics susceptibility test as a result of the heterogeneity of the strain as most of the population of organisms are susceptible to low concentrations of B-lactam antibiotics, which complicates the detection of strains resistant to low levels of antibiotic. The problem of detection of MRSA is still persistent because of the reason given above. It is in view of this that mecA gene detection has been used as an alternative way of detecting or confirming MRSA either by use of DNA probe, commercially available fluorescence test (Ieven et al., 1995), latex agglutination test (Cavassini et al., 1999), and PCR (Rohrer et al., 2001). More so, the aforementioned tests have also found to be accurate and provide results more quickly than standard susceptibility tests. PCR has been used for detection of MRSA by amplifying mecA gene in S. aureus in many laboratories especially in developed countries like USA, UK. It is in view of this, this study was aimed at determining the distribution of mecA gene in S. aureus isolates from South Western region of Nigeria by polymerase chain reaction (PCR) so as to determine the diagnostic value of using this technique for detection or confirmation of MRSA. The study found that the prevalence of mecA gene is 22% in S. aureus isolates from South Western part of Nigeria and this correlates with high level of methicillin resistance.

MATERIALS AND METHODS

Bacterial isolates

A total number of 194 strains of *S. aureus* isolated from the specimens submitted to diagnostic section of Medical Microbiology and Parasitology Department of University College Hospital (UCH), Ibadan; Obafemi Awolowo University Teaching Hospital, Ile-Ife; and LAUTECH Teaching Hospital (LTH), Osogbo; in the South Western Nigeria. The strains were isolated from different clinical specimens such as urine, blood; wound swab, ear swab, high vaginal swab and endocervical swab. The isolates were identified to be *S. aureus* following Gram stain that showed Gram positive cocci and biochemical tests showed positive results for catalase and tube coagulase as described in standard Medical Microbiology laboratory manual (Cheesbrough, 2000). All *S. aureus* isolates were stored

at 4°C on Mueller Hinton agar slope until they were ready to use.

Antibiotics susceptibility testing

The antibiotic susceptibility testing was performed using disc diffusion method on Mueller Hinton (MH) agar. Gram positive multiple antibiotic disc containing erythromycin (5 µg), chlorampehnicol (10 µg), tetracycline (10 µg), streptomycin (10 µg), penicillin (1 μg) and gentamicin(10 μg), was used. Also single antibiotic disc such as vancomycin (30µg), pefloxacin (30 µg), and ceftriazone (30 µg) were used to determine the susceptibility pattern of the isolates at 37°C overnight as previously described. Briefly, inocula of bacteria were prepared to 0.5 McFarland standards and tested against all the aforementioned antibiotics discs. Sterile swab stick was dipped into the bacteria suspension and used to streak the MH agar, after which the antibiotic disc was placed on the surface of MH agar plate and incubated at 37°C for 24 hrs. After which the zone of inhibition was measured with ruler, which was now compared with the standard value of zone of inhibition of each antibiotic. Methicillin susceptibility was determined as described before on blood agar (15% v/v) with a heavy inoculum obtained from a blood agar plate (Alli et al., 2007). The test was incubated at 30°C overnight with 10 µg/disc of methicillin. The isolates were considered sensitive. intermediate and resistant based on the guidelines of Clinical and Laboratory Standards Institute (CLSI, 2001).

Beta-lactamase test

Detection of β-lactamase was carried out using nitrocefin solution (a chromogen) on all the S. aureus isolates. Broth culture of MRSA strains as well as an ampicillin resistant strains (wild type E. coli 1048 with plasmid PUC 18) a positive control were incubated overnight, 200 µl of the overnight cultures were transferred into wells of a microtitre tray; broth without inoculum was also included to serve as a negative control. Ten microlitre of nitrocefin solution was prepared according to the manufacturer's instruction and was added to each well using a multipoint pipette. Nitrocefin is a chromogenic cephalosporin that changes colour from yellow to red on hydrolysis. β-lactamase production was inferred when the broth turned red within 30 min on addition of nitrocefin as directed by the manufacturer (Fisher Scientific, UK).

DNA Extraction and PCR for detection of *mecA* gene

DNA was extracted from 500 µl overnight Muller Hinton broth using lysostaphin and lysozyme to digest the cell wall as described before (Alli et al., 2007). PCR was carried out on all the strains using forward designated mecA F1 (AGTTCTGCAGTACCGGATTG) backward and designated mecA B1 primer (AAAATCGATGGTAAGGTTCGC) in a 30 µl reaction containing 50 ng of DNA, Taq polymerase and dNTP using the cycling parameter - denaturation temperature at 94°C for 30 sec, annealing temperature at 55°C for 30 sec, followed by extension at 72°C for 1 min for 40 cycles. Positive control (MRSA DNA) and negative control DNA from NCTC 6571 (Oxford S. aureus) were included in each batch of PCR run. A successful amplification of mecA gene would be indicated by 533 bp. Sequencing of the PCR products to confirm 533 bp mecA gene amplification was carried out on few of the positive PCR products.

Minimum Inhibitory Concentration

MIC was carried out on the selected *mecA* gene positive strains, *mecA* gene negative strains of MRSA and methicillin sensitive strains of *S. aureus*. About 12 dilutions were made using concentration covering 0.125 and 256 μg/ml of methicillin, using the original concentration as 250 μg/ml and using two fold dilution methods. Suspension of the isolates was made to obtain 0.5 MacFarland standard of the organism and 2-fold of the organism was pipette into each of the dilution to make two fold dilutions. Three controls were set up: positive control (containing MH broth and NCTC 6571 (Oxford *S. aureus* strain), negative control (containing MH broth and sterility control (containing only MH broth). The test was incubated aerobically at 37°C for 24 hrs.

Statistical Analysis

Data were analysed using statistical package within the Microsoft Excel and Epi-info software for Disease control and prevention, USA. Chi square was used to determine the effect of sex, age, hospital location, type of specimens, and beta lactamase production on the data obtained. The p value less than 0.05 was considered to be significant.

RESULTS

Among the 194 isolates of *S. aureus* obtained from different clinical specimens from 194 patients with average age of 35 years old, 45 isolates were collected from UCH, Ibadan, 58 isolates from OAUTHC, Ife, and 91 isolates from LTH, Osogbo, South Western region of Nigeria. Antimicrobial susceptibility pattern revealed that the isolates showed varying degree of resistant to various types of antibiotics tested in this study (Table 1). Highest degree of antibiotic resistant was recorded for penicillin about 186 (95.8%) out of 194 isolates of *S. aureus* whereas no resistant was found in vancomycin. The methicilin disc susceptibility testing showed that 40 (20.6%) out of 194 isolates of *S. aureus* were resistant to methicillin.

We decided to screen for *mecA* gene by PCR in all the *S. aureus* isolates irrespective of the result of methicillin susceptibility testing – a gene that has been shown to confer methicillin resistance in *S. aureus* in order to confirm the phenotypic detection of MRSA. The result of PCR (Fig. 1) showed that 43 (22.2%) out of the 194 isolates of *S. aureus* were found to contain *mecA* gene as indicated by the amplification of 533 bp expected product.

Table 1. Antibiotics susceptibility pattern of *S. aureus* isolates from South Western, Nigeria.

Antibiotic	Number of Sensitive (%)	Number of Intermediate (%)	Number of Resistant (%)	
Methicillin	154 (79.4%)	0 (0.0%)	40 (20.6%)	
Ampicillin	0(0.0%)	4 (2.2%)	190 (97.8%)	
Penicillin	0(0.0%)	8 (4.1%)	186 (95.9%)	
Streptomycin	20 (9.9%)	62 (31.9%)	102 (58.2%)	
Tetracycline	10 (5.15%)	10 (5.15%)	174 (89.7%)	
Erythromycin	60 (30.9%)	16 (8.2%)	110 (56.7%)	
Gentamicin	34 (17.5%)	28 (14.5%)	132 (68.0%)	
Chloramphenicol	42 (21.2%)	22 (11.7%)	130 (67.2%)	
Ceftriazone	28 (14.8%)	42 (21.3%)	124 (63.9%)	
Perfloxacin	48 (25.0%)	18 (9.4%)	128 (65.9%)	
Vancomycin	194 (100.0%)	0 (0.0%)	0(0.0%)	

1 2 3 4 5 6 7 8 9 10 11 12 13 M

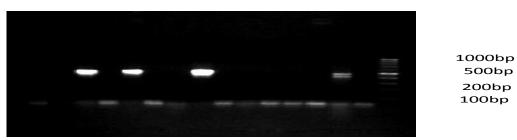


Fig 1PCR for detection of *mecA* gene among the *S. aureus* isolates. Representative agarose gel electrophoresis of PCR products. Lanes 1, 3, 6 are positive for *mecA* as indicated by 533 bp PCR product, Lane 2, 4, 5, 7-11 are negative for mecA, Lane 12: positive control; Lane 13: negative control; Lane M: molecular weight size marker.

Representatives of the positive PCR products sent for DNA sequencing confirmed the product to be part of *mecA* gene as revealed by 100% identity to the *mecA* gene (data not shown). PCR repeated for detection of *mecA* gene at higher and lower concentrations of DNA in order to rule out the effect of DNA concentration on PCR showed similar result to what obtained before. The result of the PCR confirmed phenotypic detection of MRSA with additional 3 MRSA strains detected that were not detected with disc diffusion test.

Among the 91 isolates collected from LTH, Osogbo, 20 (22%) isolates were confirmed as MRSA by detection of mecA gene, and of the 58 isolates collected from OAUTHC, Ife, 18 (31%) isolates were confirmed as MRSA by detection of mecA gene (Fig.2). There was no association between frequency distribution of MRSA and the hospitals where the isolates came from ($X^2 = 1.76$; P > 0.05).

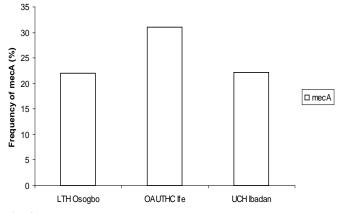


Fig.2 Distribution of *mecA* gene amongst the *S. aureus* isolates from the three teaching hospitals in South Western Nigeria.

Twenty six (22.4%) of the 116 males had MRSA while 17 (21.8%) of the 78 females had MRSA. There was no

association between gender and the frequency distribution of MRSA ($X^2 = 0.04$; P > 0.05). Age group 21-30 recorded the lowest frequency distribution (10.5%) of MRSA while the highest frequency distribution of MRSA was recorded in 51-60 age groups (Fig.3). No association was found between age group and prevalence of MRSA in South Western, Nigeria ($X^2 = 9.23$; P > 0.05).

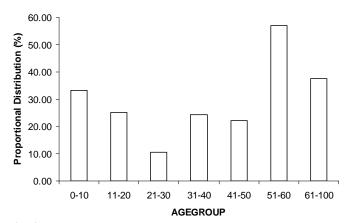


Fig. 3. Distribution of *mecA* gene in *S. aureus* according to different age groups.

The distribution of MRSA according to the specimen type / site of isolation showed that indwelling catheters and endocervical swabs accounted for 100% proportional distribution of MRSA while the lowest proportional distribution were noted in blood and nasal swabs (Table 2). There was strong association between specimen type / site of isolation of the organism and prevalence of MRSA ($X^2 = 16.74$; P < 0.05).

In order to determine the degree of methicilin resistance by the S. aureus isolates under study, the S. aureus isolates were divided into two groups - mecA positive isolate $(mecA^+)$ and mecA negative isolate

(*mecA*⁻) and minimum inhibitory concentration to methicillin was determined. The MIC₅₀ and MIC₉₀ to methicillin for representative isolates of $mecA^+$ were found to be 128 and 256 µg/ml, respectively while the MIC₅₀ and MIC₉₀ to methicillin for $mecA^-$ were found to be 0.125 and 2 µg/ml, respectively, indicating low degree of resistance to methicillin in strains without mecA gene and high degree of resistance to methicillin for strains with mecA gene.

 β –lactamase test was also carried out on the S. aureus isolates to detect whether the organism would be able to produce the enzyme β -lactamase – an enzyme that inactivates β - lactam antibiotics. The test was carried out on the 194 isolates, and 124 (64%) of the isolates were able to produce β -lactamase enzyme by changing colour from yellow to red on addition of nitrocefin solution. Among the 91 LTH isolates, 46 (50.6%) were able to produce β - lactamase, while 37 (64%) of 58 S. aureus OAUTHC isolates and 34 (75.5%) of 45 S. aureus UCH isolates were also able to produce the enzyme β -lactamase. All the 43 (100%) mecA⁺ strains showed beta lactamase activity while 81 (53.6%) of the 151 mecA strains showed beta lactamase activity. There was strong association between the presence of mecA gene and beta lactamase activity ($X^2 = 29.21$; P < 0.05).

Nasal carriage of MRSA amongst the hospital staff was also determined in order to determine the role hospital staff might play in the epidemiology of MRSA. A total of 120 samples were analysed, *S. aureus* was detected in 49 (40.4%) of the workers. Interestingly, all the *S. aureus* isolates was found to be sensitive to 10 µg methicillin disc. Furthermore, high levels of antibiotic resistant were detected among the *S. aureus* isolates from staff of the hospital.

DISCUSSION

Methicillin resistance detection in staphylococci can be problematic in the clinical microbiology laboratory because of the heterogeneity of the bacterium under test. The detection of resistance in these isolates has been troubled due to variability in standard techniques used in determining methicillin resistance. Resistant to methicillin is determined by mecA gene which is part of a mobile genetic element called the staphylococcal cassette chromosomes (SCC) mec (Wu et al., 1996), and this could be a good predictor of methicillin resistant when molecular technique such as PCR are used for screening methicillin resistant gene in S. aureus. In this present study, methicillin resistance was detected with disc susceptibility testing, minimum inhibitory concentration using broth macro-dilution method, and screening for the presence of mecA gene by PCR in all the S. aureus isolates from South Western Nigeria during the period of study. PCR for detection of mecA gene showed that 22.6% of the isolates carried the mecA gene. There was a strong correlation between high level of methicillin resistance as determined by MIC and presence of mecA gene. S. aureus isolate was considered susceptible if the MIC was less than or equal to 8 μ g/ml while \geq 16 μ g/ml as resistance, was used in interpretation of the results according to the NCCLS (Jorgensen and Turnidge, 2003).

The mecA strains of S. aureus were found to be sensitive to methicillin as indicated by MIC_{50} and MIC_{90} of 0.5 μ g/ml and 2 μ g/ml, respectively while the $mecA^+$ strains of S. aureus were 128 μ g/ml and 256 μ g/ml, respectively indicating high level of resistance to methicillin being coded for by mecA gene.

Table 2. Distribution of $mecA^+$ S. aureus according to type of specimens

Specimen type	Number of mecA+	Number of mecA-	Total	Proportional Distribution (%)
Blood	0	6	6	0.00
Catheter tip	4	0	4	100.00
Ear swab	6	8	14	42.86
Eye swab	6	20	26	23.08
Endocervical swab	2	0	2	100.00
High vaginal swab	6	12	18	33.33
Nasal swab	5	65	70	7.14
Wound swab	8	20	28	28.57
Urine	6	20	26	23.08
Total	43	151	194	22.16

This result indicates the acquisition of mecA gene is responsible for methicillin resistance; confirming other Furthermore, in order to develop high degree of resistance to methicillin, mecA is required. Studies have shown that most of the MRSA isolates contains mecA gene, while mecA is also absent in some strains of MRSA (Aires de Sousa et al., 1998). Studies have also shown that apart from mecA gene, PBP4 and ica gene cluster can also encode resistant in MRSA (Cramton et al., 1999, Memmi et al., 2008). In our study, out of the 194 isolates, 43 (22.2%) of the isolates were confirmed as MRSA by the detection of mecA gene. In support of our observation, Martin-Lopez et al. (2002) recorded 96.5% of MRSA by detection of mecA gene, which is slightly lower than the 100% detection of mecA gene in MRSA from South Western, Nigeria. A recent multicentre study in South Western Nigeria carried out by Adesida et al. (2005) confirmed resistance to methicillin by the detection of the mecA gene using PCR and reported a prevalence of 1.4 %, which is far lower to the prevalence in this study.

There is one inference that can be drawn from our study and that is mecA gene is responsible for phenotypic behaviour of methicillin resistance in this part of the world. Low level resistance to methicillin has been reported to be conferred by penicillin binding protein 4 (PBP4) ((Memmi et al., 2008). We are currently looking at this possibility among our strains of MRSA. It is also interesting to know that the MRSA nasal carriage rate was very low among the hospital workers with the non-detection of mecA gene in the entire S. aureus isolates from the hospital workers suggesting lesser role of hospital workers in dissemination of MRSA in these hospitals. More needs to be done on typing the circulating strains as previously done before in other environments using molecular epidemiological techniques (Taiwo et al., 2005, Alli et al., 2007, Okon et al., 2009) in order to monitor the epidemiology of MRSA in Nigeria. The prevalence of MRSA in this study was 22%, which is low compared to similar study carried in people living with HIV in South Africa where 77% was reported (Cotton et al., 2008), and other study in Nigeria where 34.7% was reported (Taiwo et al., 2005). Beta lactamase production by S. aureus has been identified in this study to be a risk factor for the prevalence of MRSA in South Western, Nigeria. In this study, out of the 194 isolates of S. aureus, 124 (64 %) of the isolates produce the enzyme β-lactamase, which is different from the previous study carried out by Olowe et al. (2007) in which all the isolates were found to produce β-lactamase using starch paper method. It did not come to our surprise the link between MRSA and beta lactamase production in S. aureus since the reason for

previous studies on the role of *mecA* gene in methicillin resistance (Hartman and Tomasz, 1984). the use of methicillin (beta lactamase resistant penicillin) is to combat the beta lactamase producing *S. aureus* infections. This further confirmed previous study (Alli, 1988) where majority of beta lactamase producers were methicillin resistant.

The indiscriminative use of antibiotics and because the easy availability of antibiotic without prescription has enhanced the emergency of resistant strains. Many investigators have reported an increase in the incidence of MRSA (Kim et al., 2004, Adebayo et al., 2006). Furthermore, the prevalence of MRSA in this study by the detection of mecA gene (22%) is similar to prevalence of MRSA obtained by Kesah et al. (2003) from Nigeria, which range from 21% -30%. Hospitals' location was not recognised as a risk factor in this study because no association was found between hospitals location and the prevalence of MRSA (p > 0.05). The proportional distribution of mecA gene in MRSA in this study was higher in OAU isolates (31%) compared to that of LTH isolates (22%) and UCH isolates (23%). UCH, Ibadan has been shown to be the breeding ground for multi-drug resistant bacteria as recently demonstrated by Ogbolu et al. (2011) where high degree of resistance was recorded for Gram negative enteric bacilli with no single mechanism could be used to explain the molecular basis of resistance of these isolates. Other variables such as gender and age were found not to be risk factors in MRSA infections in this study. This is in sharp contrast to a study carried out in Taiwan where age has been found to be a risk factor for S. aureus colonization (Lu et al., 2005). Interestingly, site of isolation of MRSA / specimen type has been found to be associated with prevalence of MRSA in this study; hence consider being a risk factor. High rate of isolation of MRSA had been found in indwelling devices and endocervical swabs, while low rate of isolation of MRSA had been found in blood and nasal swabs. This is in agreement with a previous study (Carnicer-Pont et al., 2006) where MRSA was found to be associated with catheter or indwelling devices.

Antimicrobial disc susceptibility pattern of the *S. aureus* isolates was determined in order to determine the multiple antibiotics resistant nature of *S. aureus* isolated in this study. Some of the *S. aureus* isolates were susceptible to ceftriazone, streptomycin, and perfloxacin. High level of resistance was recorded for penicillin despite the fact that the hospitals antibiotic usage policy in South Western, Nigeria has put a stop to the use of penicillin in treatment of infection. Although this can not rule the possibility of patients obtaining it as over the counter medicine in pharmacy shops, hence, the selective pressure on *S. aureus*

strains. Multiple antibiotic resistant has been the major property of MRSA. Antimicrobial resistance among nosocomial pathogens is a significant problem in clinical settings that adds to the cost of medical care and the morbidity and mortality of patients (Bouchillon et al., 2004). Resistance to vancomycin was found to be absent in this study. Adebayo et al (2006), recorded a 0 % resistance of vancomycin in clinical isolates of *S. aureus* collected from South Western of Nigeria, our present study corroborates that vacomycin resistant *S. aureus* is a rare event in Nigeria. This is good news, knowing fully well that vancomycin is the antibiotic of choice for treatment of MRSA.

In conclusion, the prevalence of MRSA as detected by *mecA* gene in South Western Nigeria is high enough to warrant the need for surveillance of this strain of *S. aureus* and screening of *S. aureus* for mecA gene is a good predictor of MRSA in Nigeria.

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