

ANTIBIOTIC RESISTANCE, MECA GENE DETECTION, AND BIOFILM FORMATION ABILITY AMONG COAGULASE-NEGATIVE STAPHYLOCOCCI IN CANCER PATIENTS

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ABSTRACT

Objective: The objective of the study was to identify coagulase-negative staphylococci (CoNS) from various clinical samples and to determine the antibiotic resistance of the isolates by means of automation (VITEK-2), as well as to detect biofilm formation using Congo red agar method and to detect meca gene by automated identification method (VITEK-2).

Methods: All the clinical samples (blood, urine, sputum, BAL, throat swab, wound swab, aspirated fluid, pleural fluid, and pus) received in the microbiology laboratory were processed by aseptic techniques. Clinical samples were inoculated on appropriate media (blood agar, MacConkey agar, and chocolate agar [HIMEDIA]). After inoculation, the culture plates were incubated at 37°C aerobically for 18–24 h for growth. Positive cultures were picked up and further bacterial species identification was done using automated techniques (MALDI-TOF).

Results: Among 28 isolates, the most recurrent strains of CoNS are *Staphylococcus epidermidis*, *Staphylococcus hominis*, *Staphylococcus lugdunensis*, and *Staphylococcus haemolyticus*. The assessment of antibacterial drugs sensitivity shows that all the isolates were more sensitive to daptomycin (*S. epidermidis* 100%, *S. hominis* 100%, *S. lugdunensis* 100%, and *S. haemolyticus* 42.85%) followed by linezolid (*S. epidermidis* [69.23%], *S. hominis* [100%], *S. lugdunensis* [100%], and *S. haemolyticus* [57.14%]) and vancomycin (*S. epidermidis* [100%], *S. hominis* [40%], *S. lugdunensis* [100%], *S. haemolyticus* [42.85%]). The analysis revealed the presence of the meca gene (67.85%) and biofilm production (85.71%), respectively.

Conclusion: Our data indicate that the hospital environment can be colonized by biofilm forming CoNS and transmission of these strains can cause an increased risk of serious nosocomial infections.

Keywords: Coagulase-negative bacteria, Biofilm, Nosocomial infection.

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INTRODUCTION

Staphylococcus aureus is a common source of nosocomial infection in hospitals. A wide variety of pathogens is highly laden in hospital environment and the most frequent nosocomial infections are infections of surgical wounds, urinary tract infections, and lower respiratory tract infections. Staphylococci are the important pathogenic bacteria responsible for a variety of diseases in humans and other animals [1]. Staphylococci are usually divided into two groups based on their ability to produce coagulase, an enzyme that clots blood plasma. The most common pathogen in the genus is coagulase-positive *S. aureus*, which causes deep infections such as septic arthritis, endocarditis, osteomyelitis, pneumonia, and skin infections. Several nosocomial infections such as heart valves, pacemaker lines, boxes, cerebrospinal shunts, and prosthetic joints are considered as significant pathogens of coagulase-negative staphylococci (CoNS).

Methicillin is a narrow-spectrum beta-lactam antibiotic of the penicillin class developed in 1959 that was previously used to treat infections caused by beta-lactamase-producing *S. aureus*. Methicillin has recently been renamed methicillin to comply with European law [2]. Methicillin resistance has emerged in most strains of the bacteria (*Staphylococcus epidermidis*, *S. saprophyticus*, *Staphylococcus haemolyticus*, *Staphylococcus hominis*, and another 49 species), and they are known as methicillin-resistant CoNS (MR-CoNS), is a major health concern. In *Staphylococcus* species, methicillin resistance results from the recombinase-mediated insertion of a mobile genetic element that carries meca and various antibiotic resistance genes called *Staphylococcal* chromosomal cassette mec, the acquisition of the meca gene, which produces a mutated penicillin-binding protein 2a with a

low binding affinity for all beta-lactam antibiotics, causes resistance in MRSA and MR-CoNS. The potent inducer of the meca regulatory system is cefoxitin and it is widely used as a surrogate marker for the detection of meca gene-mediated methicillin resistance [1]. Multicellular communities of bacteria are called biofilms. Various biotic and abiotic surfaces are attached to an extracellular polymeric matrix produced by the bacteria [3].

CoNS are becoming more common as medical research advances, particularly with the increased use of medical equipment. It is critical to characterize CoNS at the species level, as well as their antimicrobial capabilities and biofilm development, to diagnose and treat it. As a result, the current investigation will be studied to establish the species distribution, antimicrobial susceptibility, biofilm formation, and meca gene detection of CoNS causing infections in cancer patients at our center.

METHODS

The study was conducted among cancer patients in the microbiology division of a tertiary cancer center. It is a hospital-based cross-sectional study that was carried out among 645 specimens from medical, surgical, and allied superspecialty units. The factors such as sex, age, date of admission, and diagnosis were also recorded. This study was carried out for 3 months from June 2021 to August 2021.

Sample collection

All the clinical samples (blood, urine, sputum, BAL, throat swab, wound swab, aspirated fluid, pleural fluid, and pus) received in microbiology laboratory during the study period were taken for the study.

Specimens received in the microbiology laboratory were subjected to Gram stain followed by inoculation on blood agar and Chocolate agar aseptically. The inoculated culture plates were incubated at 37°C for 18–24 h. After reading and interpreting the culture results, the clinically significant isolates were identified using standard methods.

Diagnostic criteria for the identification of isolates

The isolates were identified by Gram stain, colony morphology and species identification were done by MALDI-TOF. Antimicrobial susceptibility was done following CLSI guidelines. Biofilm formations were detected using Congo red agar method.

Antimicrobial susceptibility testing by VITEK 2

VITEK2 is a fully automated system for bacterial identification and susceptibility testing using fluorescence-based technology. For organism identification, fluorogenic methodology is used and for susceptibility testing, turbidimetric methods used by a 64-well card that is barcoded with information on card type, expiration date, lot number, and unique card identification number [4].

Bacteria which showed methicillin resistance were checked for *mecA* gene detection with VITEK2 [5].

Biofilm production by Congo red agar (CRA) method

CRA method is a qualitative assay for the detection of biofilm producer microorganism, as a result of color change of colonies inoculated on CRA method. All the CoNS were subjected for biofilm production by CRA method [6].

RESULTS AND OBSERVATIONS

A total of 645 specimens were collected in the Microbiology Division of Malabar Cancer Centre during the study period out of which 152 (23.5%) isolates showed culture positive (Table 1 and Fig. 1).

Microbial etiology of specimens was studied, based on groups, Gram-positive cocci (GPC) were found as 64 (42.10%) (Table 2 and Fig. 2).

Among GPC, 28 (43.75) were CoNS. *S. epidermidis* were responsible for 13 (46.42%) of infections, followed by *S. haemolyticus* 7 (25%), *S. hominis* 5 (17.85%), and *S. lugdunensis* 3 (10.71) (Table 3 and Fig. 3).

CoNS can cause infection at any age, however, prevalence was high among elderly age group (41–50 age) which showed 25% (Table 4 and

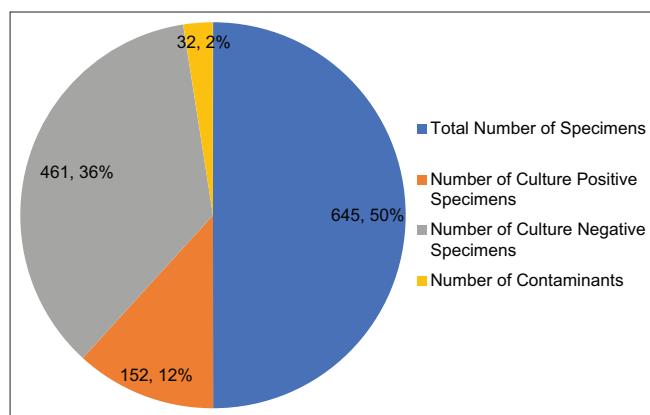


Fig. 1: Culture results of specimens

Table 1: Culture results of specimens

Total number of specimens	Number of culture-positive specimen (%)	Number of culture-negative specimens (%)	Number of culture contaminants (%)
645	152 (23.5%)	461 (71.4%)	32 (4.9%)

Fig. 4). Among sex-wise distribution, males showed high prevalence (57.1%) compared to females (Table 5 and Fig. 5).

We had maximum CoNS in pus sample 39.28%, followed by blood 25%, axillary swab 14.28%, urine 7.14%, nasal swab 7.14%, and sputum 7.14% (Table 6 and Fig. 6).

Antibiotic susceptibility pattern of all CoNS showed that all *mecA* gene producers (*S. epidermidis*, *S. hominis*, *S. haemolyticus*, and *S. lugdunensis*) were multidrug resistant. However, significant isolates *S. epidermidis* 100%, *S. hominis* 100%, *S. haemolyticus* 42.85%, and *S. lugdunensis* 100% were more susceptible to daptomycin followed by linezolid (*S. epidermidis* 69.23%, *S. hominis* 100%, *S. haemolyticus* 57.14%, and *S. lugdunensis* 100%) and for vancomycin, the susceptibility was *S. epidermidis* 100%, *S. hominis* 40%, *S. haemolyticus* (42.85%), and *S. lugdunensis* (100%) (Table 7). However, the study shows that from the antibiotic tested, daptomycin can be the drug of choice.

All the isolates were looked for *mecA* gene detection, among which 67.85% showed positive for *mecA* gene (Table 8).

Among biofilm producers, 58.33% were *S. epidermidis*, followed by *S. haemolyticus* 16.66%, *S. hominis* 12.5%, and *S. lugdunensis* 12.5% (Table 9 and Fig. 7).

DISCUSSION

CoNS are opportunistic pathogens that are a common cause of nosocomial infections. Because these organisms were a normal flora on the skin, they were long regarded non-pathogenic. However, from recent two decades, they are considered as significant pathogens due to increase in medical devices. In addition, CoNS have started to develop resistance to various antimicrobial agents which are difficult to treat.

In the present study out of 64 (42.10%) GPC isolated, 28 were CoNS. Using MALDI-TOF, the isolates were identified as *S. haemolyticus*, *S. lugdunensis*, *S. epidermidis*, and *S. hominis*. The findings of the present study were in agreement with various studies which showed *S. epidermidis* as the most common coagulase-negative staphylococci. In contrast, a finding of Jain et al. [7] showed that the most common pathogenic isolate were *S. haemolyticus* (58%) as followed by *S. epidermidis* (19%).

The underlying risk factors which are associated with CoNS infection include age. In our study, 41–50 age groups showed more prevalence. A similar study showed that CoNS infection was maximum at the age group between 21 and 30, that is, 25%.

Our study also showed that males (57.1%) were more infected than females (42.85%). These results were in consistent with a study Munda et al., 2009 [8], which showed out of 60 coagulase negative Staphylococcus reported 53.33% were reported in males while the remaining 46.67% were in females [9].

In our study, maximum number of CoNS were observed in pus samples (39.28%). A similar study from Haryana showed that maximum number of CoNS were isolated in urine samples (41.75%). The antimicrobial susceptibility of the isolates was tested against 13 antimicrobial drugs in accordance with CLSI guidelines using VITEK2.

Antimicrobial susceptibility pattern of the isolates showed that daptomycin was more susceptible (*S. epidermidis* 100%, *S. hominis* 100%, *S. lugdunensis* 100%, and *S. haemolyticus* 42.85%) for all the isolates

Table 2: Microbial etiology of specimens

Pathogens	Total number of isolates (n=152)	Percentage
Gram-positive cocci	64	42.10
Gram-negative bacilli	86	56.57
Budding yeast	2	1.31

Table 3: Microbial etiology of coagulase-negative *Staphylococci*

Pathogens	Total numbers	Percentage
<i>S. epidermidis</i>	13	46.42
<i>S. haemolyticus</i>	7	25
<i>S. hominis</i>	5	17.85
<i>S. lugdunensis</i>	3	10.71
Total	28	100

S. epidermidis: *Staphylococcus epidermidis*, *S. hominis*: *Staphylococcus hominis*,
S. lugdunensis: *Staphylococcus lugdunensis*, *S. haemolyticus*: *Staphylococcus haemolyticus*

Table 4: Age distribution of patients with coagulase-negative *Staphylococci*

Age	Number of patients (n=28)	Percentage
0-10	5	17.85
11-20	1	3.57
21-30	2	7.14
31-40	1	3.57
41-50	7	25
51-60	6	21.42
>60	6	21.42
Total	28	100

Table 5: Sex-wise distribution of patients with coagulase-negative *staphylococci* infection

Pathogen (n=28)	Male	Female	Total
<i>S. epidermidis</i>	8	5	13
<i>S. haemolyticus</i>	3	4	7
<i>S. hominis</i>	3	2	5
<i>S. lugdunensis</i>	2	1	2
Total (%)	16 (57.1)	12 (42.85)	28 (100)

S. epidermidis: *Staphylococcus epidermidis*, *S. hominis*: *Staphylococcus hominis*,
S. lugdunensis: *Staphylococcus lugdunensis*, *S. haemolyticus*: *Staphylococcus haemolyticus*

tested. The result of our study is consistent with another research article, Uekötter et al., 2011 [10], however, certain studies showed that vancomycin drug to be more sensitive, Natoli et al. [11], observed no resistance to vancomycin. The methicillin-resistant rate in our study was 67.85%, however, the methicillin resistance of CoNS isolated was reported high in Brazil (77%), Colombia (73%), and Egypt (75%), which could be over use of antibiotics which have resulted in CoNS colonization. A study from Malaysia showed that misintreption of contaminated cultures let to the emergence of vancomycin resistance staphylococci.

Out of 28 CoNS, 19 were methicillin-resistant staphylococci, similar result 39.4% were found by Khadri and Alzohairy, 2010 [12]. We have observed that the resistant rate to different antibiotics among MR-CoNS strains was higher than those sensitive to methicillin and this finding was similar to Sangwan and Kumari, 2018 [13].

The present study also reveals that 24 isolates were biofilm producers, they were more resistant to antimicrobial agents compared to non-biofilm producers. This result was more similar to the findings reported by Martineau et al., 2016 [9], who found that 90.8% of MR-CoNS were biofilm producers.

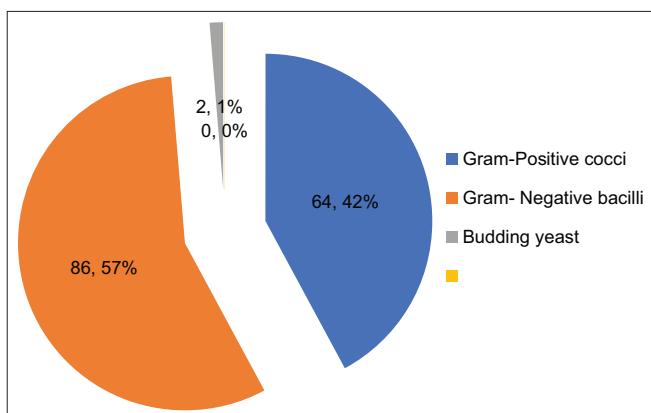
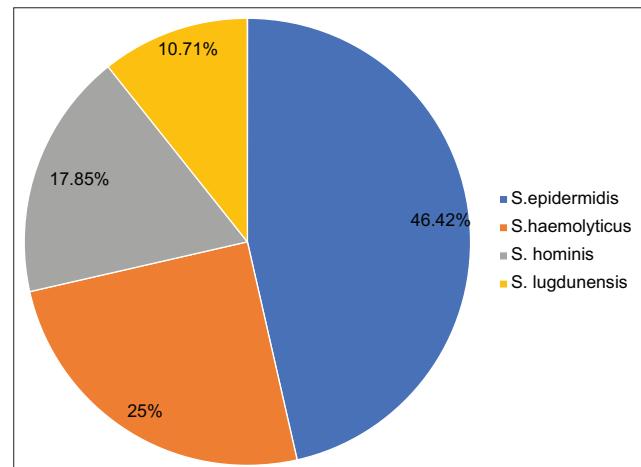
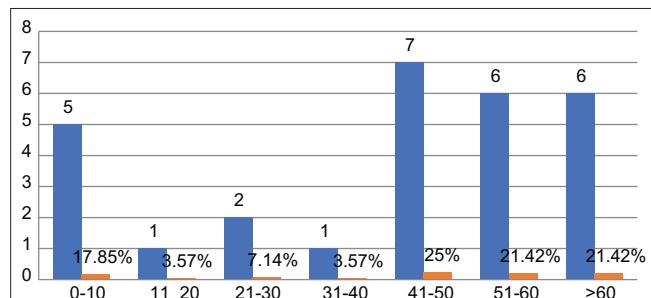
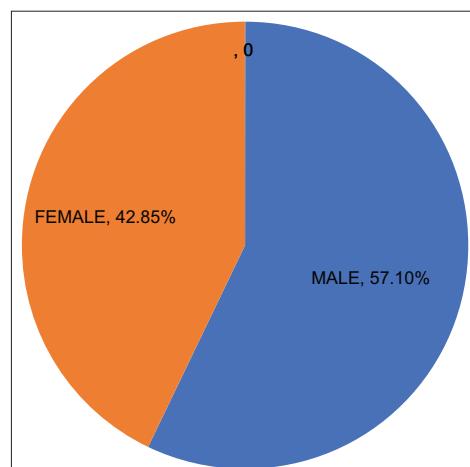
**Fig. 2: Microbial etiology of specimens: Organism based on groups****Fig. 3: Microbial etiology of coagulase-negative staphylococci****Fig. 4: Age distribution of patients with CoNS infection****Fig. 5: Sex-wise distribution of patients with CoNS infection**

Table 6: Distribution of coagulase-negative *staphylococci* species in various clinical samples

Organisms	Urine	Blood	PUS	Nasal swab	Sputum	Axillary swab
<i>S. epidermidis</i>	2	1	8	2	2	3
<i>S. haemolyticus</i>	-	2	1	-	-	-
<i>S. hominis</i>	-	4	-	-	-	1
<i>S. lugdunensis</i>	-	-	2	-	-	-
Total (%)	2 (7.14)	7 (25)	11 (39.28)	2 (7.14)	2 (7.14)	4 (14.28)

S. epidermidis: *Staphylococcus epidermidis*, *S. hominis*: *Staphylococcus hominis*, *S. lugdunensis*: *Staphylococcus lugdunensis*, *S. haemolyticus*: *Staphylococcus haemolyticus*

Table 7: Antibiotic susceptibility testing of coagulase-negative *staphylococci*

Antibiotics	<i>S. epidermidis</i>		<i>S. hominis</i>		<i>S. haemolyticus</i>		<i>S. lugdunensis</i>	
	S	R	S	R	S	R	S	R
Ciprofloxacin	15.38	84.62	20	80	57.14	42.85	50	50
Clindamycin	38.46	61.54	20	80	42.85	57.14	50	50
Daptomycin	100		100		42.85	57.14	100	
Erythromycin	38.46	61.54	-	-	-	-	50	50
Gentamicin	61.53	38.47	40	60	28.57	71.43	50	50
Levofloxacin	15.38	84.62	20	80	57.14	42.85	50	50
Rifampicin	46.15	53.85	20	80	42.85	57.14	50	50
Linezolid	69.23	30.77	100		57.14	42.85	100	
Teicoplanin	100		20	80	42.85	57.14	50	50
Tetracycline	53.84	46.16	20	80	42.85	57.14	50	50
Tigecycline	46.15	53.85	40	60	42.85	57.14	100	
Trimethoprim	30.76	69.24	60	40	28.57	71.43	50	50
Vancomycin	100		40	60	42.85	-	100	

CIP: Ciprofloxacin, CD: Clindamycin, DAP: Daptomycin, E: Erythromycin, GEN: Gentamicin, TE: Tetracycline, RIF: Rifampicin, LIN: Linezolid and SXT trimethoprim/sulfamethoxazole, LEV: Levofloxacin, TEC: Teicoplanin, TGC: Tigecycline, VA: Vancomycin. *S. epidermidis*: *Staphylococcus epidermidis*, *S. hominis*: *Staphylococcus hominis*, *S. lugdunensis*: *Staphylococcus lugdunensis*, *S. haemolyticus*: *Staphylococcus haemolyticus*

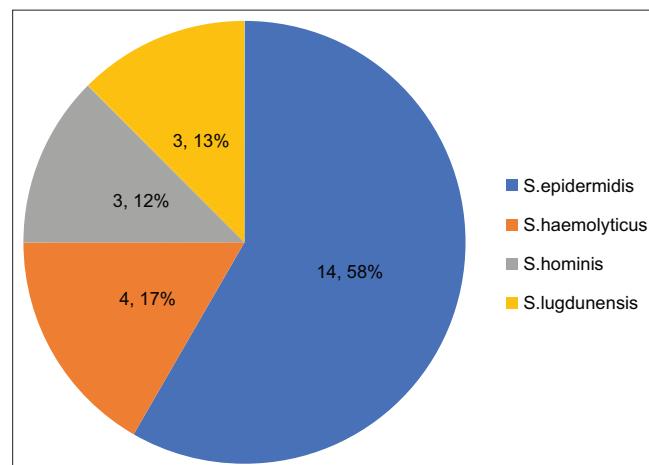
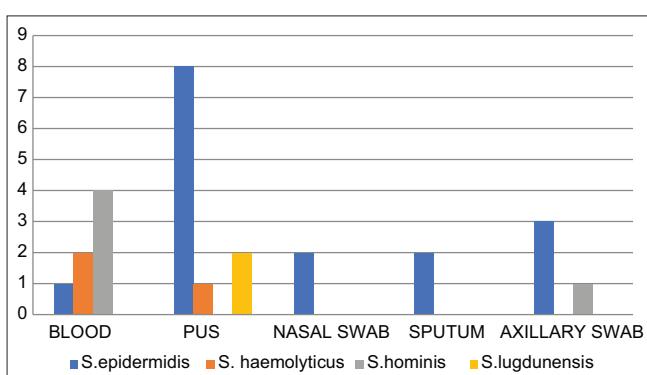
Table 8: Percentage of isolates positive for *mecA* gene

Total number of isolates	Percentage
19	67.85%

Table 9: Biofilm producers

Organisms	Number of biofilm producers	Percentage
<i>S. epidermidis</i>	14	58.33
<i>S. haemolyticus</i>	4	16.66
<i>S. hominis</i>	3	12.5
<i>S. lugdunensis</i>	3	12.5
Total	24	100

S. epidermidis: *Staphylococcus epidermidis*, *S. hominis*: *Staphylococcus hominis*, *S. lugdunensis*: *Staphylococcus lugdunensis*, *S. haemolyticus*: *Staphylococcus haemolyticus*

**Fig. 7: Biofilm producers****Fig. 6: Distribution of coagulase-negative staphylococci species in various clinical samples**

CONCLUSION

The present study showed the emergence and occurrence of CoNS causing infection in cancer patients at Malabar Cancer Centre, Thalassery.

A total of 28 CoNS were studied, in which four species were identified; *S. epidermidis* 46.42% were the most common species followed by *S. haemolyticus* 25%, *S. hominis* 17.85%, and *S. lugdunensis* 10.71%.

Antimicrobial sensitivity pattern of CoNS documented showed *S. epidermidis* (100%), *S. hominis* (100%), *S. lugdunensis* (100%), and *S. haemolyticus* (42.85%) higher sensitivity to daptomycin followed by linezolid (*S. epidermidis* [69.23%], *S. hominis* [100%], *S. lugdunensis* [100%], and *S. haemolyticus* [57.14%]).

mecA gene detection and biofilm production were detected in 67.85% and 85.71%.

All the biofilm forming strains isolated were multidrug resistant. CRA method is used to detect biofilm production in 28 isolates. It showed biofilm positivity for 24 isolates. In the previous study, similar results were obtained. A study by Shrestha *et al.*, 2012, showed that the susceptibility, specificity, and accuracy of the CRA method for biofilm production were higher than tube adherence method. Due to decreasing antibiotic sensitivity and host immune response, it is difficult to treat biofilm-producing staphylococci clinically. This severely limits to the treatment option for an effective therapy. Keeping in mind, the increasing prevalence of patients being infected with CoNS rapid diagnosis of these strains in microbiology laboratories is critical to implement proper antibiotic use and infection control strategies.

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Nil.

AUTHORS' CONTRIBUTIONS

Dr. Sajani Samuel, Ms. Dilsha TK, and Dr. Parthiban R contributed substantially to the conception, design of the study, analysis, and interpretation of data. All authors discussed the results and commented on the manuscript. Dr. Sajani Samuel and Dr. Parthiban R drafted the final manuscript.

CONFLICTS OF INTEREST STATEMENT

None to declare.

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Nil.

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