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RESEARCH ARTICLE

ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF STAPHYLOCOCCUS AUREUS ISOLATED FROM VARIOUS CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL

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Abstract

Background:

Staphylococcus aureus is a nosocomial pathogen, causing high morbidity and mortality. The prevalence of MRSA infection and the susceptibility pattern of Staphylococcus aureus varies with the region.

With this background, present study was undertaken to determine the prevalence of staphylococcus aureus and Methicillin-Resistant Staphylococcus aureus (MRSA) isolates from various clinical samples and their antimicrobial susceptibility pattern in a tertiary care hospital.

Materials and Methods:

In this retrospective study, a total of 240 isolates of Staphylococcus aureus was collected from a clinical specimen, between June 2021 to May 2022 in the Department of Microbiology, MGM Medical College and Hospital, Aurangabad. Staphylococcus aureus was identified and their antibiotic susceptibility testing was determined by using Vitek 2 compact system and data were analyzed.

Result:

In our study, total of 240 Staphylococcus aureus were isolated and the prevalence of MSSA was 27.5% and the prevalence of MRSA was 72.5% from various clinical samples.

Out of 240 Staphylococcus aureus isolates, 116 (48.33%) were resistant to Benzylpenicillin, followed by Ciprofloxacin 212 (88.33%), Levofloxacin 212 (88.33%), Erythromycin 154 (64.17%), Clindamycin 122 (50.83%), Trimethoprim/Sulfamethoxazole 120 (50.00%), Gentamicin 56 (23.33%), Rifampin 15 (6.25%) and Tigecycline 6 (2.50%).

Conclusion:

A high prevalence of MRSA (72.50%) was observed in our region. The most effective antibiotics for the treatment of MRSA were Linezolid, Vancomycin, Teicoplanin, and Tetracycline. These antibiotics should be kept reserved for the treatment of MRSA infection.

Regular surveillance of hospital-associated infections is required for assessing the trends in the antibiotic resistance of pathogens for strengthening the control measures to prevent the spread and reduce the emergence of multidrug-resistant MRSA infections.

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Introduction:-

Staphylococcus aureus has been found to be the most clinically important species, with a wide presence in nature. It is part of the normal flora of the human body and generally carried on the skin or in the nose of healthy people, which makes it simple to be transmitted through hair or fomites from patients or carriers [1].

It is one of the most important pathogens affecting humans, and causes a wide range of infections from skin wounds and deep tissue infections to life-threatening pneumonia, endocarditis, septic arthritis, and septicemia [2].

Staphylococcus aureus is an important pathogen of many nosocomial and community-based infections causing high morbidity and mortality [3].

Methicillin-

Resistant Staphylococcus aureus (MRSA) is an MDR strain of Staphylococcus aureus, resistant to penicillin, cephalosporins, carbapenems, and macrolides. This causes serious endemic and epidemic MRSA infections [4].

Various studies from different parts of the world show an increase in the number of Methicillin-resistant Staphylococcus aureus (MRSA) [5,6,7]. The prevalence of Methicillin-resistant Staphylococcus aureus (MRSA) in India ranges from 30–70%. 30% in the northeast part of India to 50% in south India [8,9,10,11].

Since MRSA is resistant to all β -lactam antibiotics, the therapeutic options are limited significantly, and therefore their accurate identification becomes important for the practicing physicians, clinical microbiologists, and public health officials, knowledge of the local antimicrobial resistance patterns of MRSA is essential to guide empirical and pathogen-specific antibiotic therapy and also is critical for taking optimal decisions in infection control policies [12,13].

The present study was undertaken to determine the prevalence of staphylococcus aureus and Methicillin-resistant Staphylococcus aureus (MRSA) isolates from various clinical samples and their antimicrobial susceptibility pattern in a tertiary care hospital.

Material and Method:-

The present study was a Retrospective, cross-sectional study conducted in the Department of Microbiology, MGM Medical College and Hospital, Aurangabad. The duration of the study was from June 2021 to May 2022 and was approved by the institutional ethics committee. The susceptibility data of Staphylococcus aureus isolates in the study period was collected and analysed. The data included the patient's location, source of isolate, and antibiotic susceptibility pattern.

A total of 240 Staphylococcus aureus isolates were collected from different clinical samples from the outpatient department (OPD), inpatient department (IPD), and ICU like Pus, Blood, Urine, Sputum, and Endotracheal secretion (ET). All specimens were processed as per standard Bacteriological protocol. The specimen was inoculated on Blood agar & MacConkey agar and incubated at 37°C overnight.

Staphylococcus aureus was identified by its characteristic golden yellow with beta-hemolytic colonies on blood agar, Gram stain showing Gram-positive cocci in clusters, mannitol fermentation, and positive by catalase, slide, and tube coagulase methods [14]

A bacterial suspension was prepared and processed as per the manufacturer's instruction (BioMerieux) [15]. Staphylococcus aureus was identified by using Vitek 2 compact system (BioMerieux) [15]. The final results were obtained and interpreted by the ID-GPC database. For Quality control, Staphylococcus aureus ATCC strain 29213 was used.

The antibiotic susceptibility pattern of Staphylococcus aureus was also interpreted from the VITEK-2 system. Isolates for which cefoxitin screen was positive and oxacillin MIC was $\geq 4 \mu\text{g/ml}$ were regarded as MRSA as per CLSI guidelines.

Isolates for which cefoxitin screen was negative and oxacillin MIC was $\leq 2 \mu\text{g/ml}$ were regarded as MSSA as per CLSI guidelines. The antibiotic tested included Benzylpenicillin, Gentamicin, Ciprofloxacin, Levofloxacin, Erythromycin, Rifampicin, Clindamycin, Trimethoprim/Sulfamethoxazole, Vancomycin, Linezolid, Teicoplanin, Tetracycline, and Tigecycline.

The Vitek 2 results were reported as susceptible (S), intermediate (I), or resistant (R) according to CLSI guidelines. All data were entered in an excel sheet and the data was statistically analysed.

Result:-

A total of 240 *Staphylococcus aureus* were studied. Out of the 240 isolates of *Staphylococcus aureus*, 66 (27.5%) were MSSA, and 174 (72.5%) were MRSA (Figure No.1).

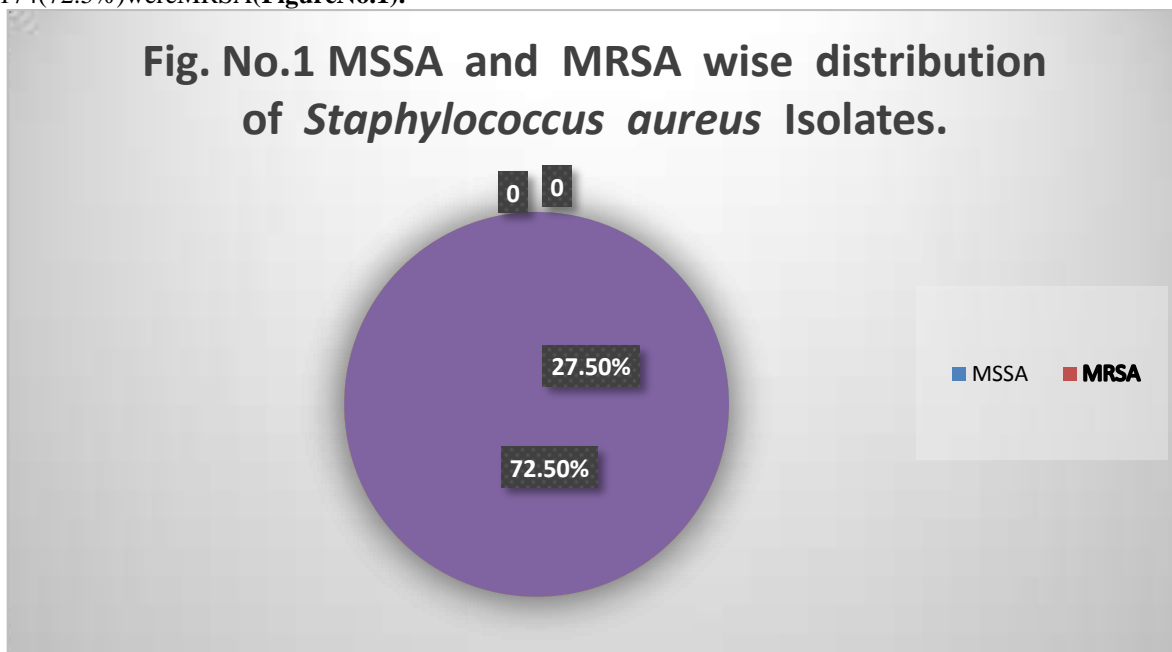


Figure No.1:-

The majority of the isolates were obtained from the inpatient Department followed by ICU and outpatient Departments. (Table No.1)

Table No.1	Patients' wise distribution of MSSA and MRSA Isolates.		
Type of Patients	MSSA (n=66) (n=%)	MRSA (n=174) (n=%)	Total (n=240) (n=%)
OPD	6 (9.09%)	33 (18.97%)	39 (16.25%)
IPD	35 (53.03%)	96 (55.17%)	131 (54.58%)
ICU	25 (37.88%)	45 (25.86%)	70 (29.17%)

Table No.2	Gender-wise distribution of MSSA and MRSA Isolates.		
Gender	MSSA (n=66) (n=%)	MRSA (n=174) (n=%)	Total (n=240) (n=%)
Male	40 (60.61%)	102 (58.62%)	142 (59.17%)
Female	26 (39.39%)	72 (41.38%)	98 (40.83%)

Out of the 240 isolates of *Staphylococcus aureus*, 142 (59.17%) were isolated from Male patients and 98 (40.83%) were isolated from Female patients. (Table No.2)

TableNo.3		Specimen-wisedistributionofMSSAandMRSAIsolates.		
Sr.No.	Specimen	MSSA(n=66)(n=%)	MRSA(n=174)(n=%)	Total(n=240)(n=%)
1.	Pus	42(63.64%)	124(71.26%)	166(69.17%)
2.	Blood	14(21.21%)	34(19.54%)	48(20.00%)
3.	ETsecretion	6(9.09%)	12(6.90%)	18(7.50%)
4.	Sputum	2(3.03%)	3(1.72%)	5(2.08%)
5.	Urine	2(3.03%)	1(0.57%)	3(1.25%)

Outof240Staphylococcus aureusisolatesweremainlyisolatedfromskinandsofttissueinfections suchaspus166(69.17%) followedbyblood48(20%),endotrachealsecretions18(7.50%),sputum5(2.8%)andurine3(1.25%).(TableNo.3)

TableNo.4		AntimicrobialsensitivitypatternofMSSAandMRSAtothevariousantibiotics.					
Sr.No.	NameofAntibiotics	MSSA(n=66)(n=%)			MRSA(n=174)(n=%)		
		Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
1	Gentamicin	60(90.91%)	--	6(9.09%)	76(43.68%)	48(27.59%)	50(28.74%)
2	Ciprofloxacin	12(18.18%)	12(18.18%)	42(63.64%)	4(2.30%)	--	170(97.70%)
3	Benzylpenicillin	24(36.36%)	--	42(63.64%)	--	--	174(100%)
4	Rifampicin	--	--	--	60(34.48%)	--	15(8.62%)
5	Clindamycin	42(63.64%)	--	24(36.36%)	76(43.68%)	--	98(56.32%)
6	Levofloxacin	12(18.18%)	12(18.18%)	42(63.64%)	4(2.30%)	--	170(97.70%)
7	Erythromycin	30(45.45%)	6(9.09%)	30(45.45%)	40(22.99%)	--	124(71.26%)
8	Trimethoprim/Sulfamethoxazole	42(63.64%)	--	24(36.36%)	78(44.83%)	--	96(55.17%)
9	Linezolid	66(100%)	--	--	174(100%)	--	--
10	Vancomycin	66(100%)	--	--	174(100%)	--	--
11	Tetracycline	66(100%)	--	--	174(100%)	--	--
12	Tigecycline	66(100%)	--	--	168(96.55%)	--	6(3.45%)
13	Teicoplanin	66(100%)	--	--	174(100%)	--	--

The majority of the 70(29.17%) Staphylococcus aureus isolates were obtained from ICU and different wards such as Surgery 78(32.50%) followed by Medicine 42(17.50%), Nephrology 30(12.50%), OBGY 8(3.33%), ENT 7(2.92%), Ophthalmology 3(1.25%) and Orthopaedics 2(0.83%) wards.

Out of 66(27.5%), MSSA isolates all were sensitive to Linezolid, Vancomycin, Tetracycline, Tigecycline, and Teicoplanin, followed by Gentamicin 60(90.91%), Clindamycin 42(63.64%), Trimethoprim/Sulfamethoxazole 42(63.64%), Erythromycin 30(45.45%), Benzylpenicillin 24(36.36%), Ciprofloxacin 12(18.18%) and Levofloxacin 12(18.18%).

Out of 66(27.5%), MSSA isolates 12(18.18%) were intermediate sensitive to Ciprofloxacin, Levofloxacin, and Erythromycin 6(9.09%).

Out of 66(27.5%), MSSA isolates 42(63.64%) were resistant to Ciprofloxacin, Benzylpenicillin, and Levofloxacin, followed by Erythromycin 30(45.45%), Clindamycin 24(36.36%), Trimethoprim/Sulfamethoxazole 24(36.36%) and Gentamicin 6(9.09%).

Out of 174 (72.5%), MRSA isolates all were sensitive to Linezolid, Vancomycin, Tetracycline, and Teicoplanin, followed by Tigecycline 168 (96.55%), Trimethoprim/Sulfamethoxazole 78 (44.83%), Gentamicin 76 (43.68%), Clindamycin 76 (43.68%), Rifampicin 60 (34.36%), Erythromycin 40 (22.99%), Ciprofloxacin 4 (2.30%) and Levofloxacin 4 (2.30%).

Out of 174 (72.5%), MRSA isolates 48 (27.59%) were intermediate sensitive to Gentamicin.

Out of 174 (72.5%), MRSA isolates all were resistant to Benzylpenicillin, followed by Ciprofloxacin 170 (97.70%), Levofloxacin 170 (97.70%), Erythromycin 124 (71.26%), Clindamycin 98 (56.32%), Trimethoprim/Sulfamethoxazole 96 (55.17%), Gentamicin 50 (28.74%), Rifampicin 15 (8.62%) and Tigecycline 6 (3.45%). (**Table No. 4**)

There was no resistance documented against Linezolid, Vancomycin, Tetracycline, and Teicoplanin. All the 122 Clindamycin resistant isolates, 85 were inducible Clindamycin resistance. The antimicrobial sensitivity testing of MRSA and MSSA show that MRSA is resistant to most antibiotics as compared to MSSA.

Discussion:-

Methicillin-

resistant *Staphylococcus aureus* (MRSA) has caused septicaemia, pneumonia, wound infections, septic arthritis, osteomyelitis, toxic shock syndrome, and hospital-acquired as well as community-acquired infections, with significant rates of morbidity and mortality [16]

The rapid evolution of antibiotic resistance in *Staphylococcus aureus* is of considerable concern. Among Gram-positive bacteria, *Staphylococcus aureus* is known for resistance to various commonly used antibiotics. Therefore, accurate identification is needed not only for choosing the appropriate antibiotic but also to control the endemicity of MRSA. MRSA (Methicillin-resistant *Staphylococcus aureus*) is a serious public health problem and an economic burden on national health care systems.

In our study, out of 240 isolates of *Staphylococcus aureus*, the majority of the isolates were obtained from pus samples 166 (69.17%), which formed the largest group of samples followed by Blood 48 (20.00%), ET secretion 18 (7.50%), sputum 5 (2.08%) and urine 3 (1.25%). A similar type of pattern was also observed by Sapkota et al. [17]. The study was conducted in Tamil Nadu by V. Vasuki and S. Ananthasankari et al. also showed that pus & wounds abscess accounted for the majority (57.8%) of isolates followed by sputum, blood, urine, and CSF [13].

Similarly, Himani et al. has also reported that the majority of *Staphylococcus aureus* isolates were from pus (78.53%) followed by endotracheal secretion, urine & body fluids [18]. On the contrary, Sajina et al. in their study isolated the maximum number of *S. aureus* from urine (39.6%) followed by blood (16.7%), sputum (20.8%) pus (13.2%) & Body fluid (0.7%) [4].

In our study, out of 240 *Staphylococcus aureus* isolates, 66 (27.5%) were MSSA, and 174 (72.5%) were MRSA. Similarly, Sapkota et al. reported 70.64% MRSA and 29.36% MSSA isolates in their study [17]. Likewise, Shilpa Arora et al. in their study conducted in the Department of Microbiology, Govt. Medical College, Amritsar, India has also reported 135 (56%) MSSA and 115 (46%) were MRSA isolates [9].

L. K. Khan et al. observed that in 600 isolates of *Staphylococcus aureus*, 192 (32.00%) were MSSA, and 408 (68.00%) were MRSA in their study [19]. Nabamita Chaudhury et al. observed that in 358 isolates of *Staphylococcus aureus*, 151 (45.18%) were MSSA, and 207 (57.82%) MRSA in their study was conducted in West Bengal, India [20].

Likewise, Geeta S. H. et al. in their study conducted in Bangalore, India has also reported 166 (72.29%) MSSA and 44 (26.51%) MRSA isolates [12]. Abdel-

Aty E. Alfy et al. observed that out of 170 isolations of *Staphylococcus aureus*, 32 (18.08%) were MSSA and 138 (81.02%) were MRSA in their study from Egypt [21]. Himani et al. has also reported that 66 (40.4%) isolates of *Staphylococcus aureus* were MRSA [18].

Sanjeev Sahai et al. observed the isolation of 31% MRSA in their study from Uttar Pradesh [4]. The highest isolation of MRSA was observed in a tertiary care hospital in Kerala by Anitha Madhavan et al. who reported the isolation of 72% of MRSA, which is much higher than our study [22].

Likewise, Sangeeta Joshi et al. has reported higher isolation of MRSA (53%) in their study from the Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group, India [23]. On the contrary, V. Vasuki & S. Ananthasankari et al. in their study have observed that only 26 (57.8%) of *S. aureus* isolates were found to be MRSA which is much lower than our study [13]. Similarly, Sanjeev Saha et al. from Chandigarh reported a low 31 (31%) isolation of MRSA in their study [4].

The MRSA isolates were associated with a high level of co-resistance to other groups of antimicrobial agents.

In our study, out of 66 (100%), MSSA isolates all were sensitive to Linezolid, Vancomycin, Tetracycline, Teicoplanin, followed by 60 (90.91%) sensitive to Gentamicin, 12 (18.18%) were sensitive to Ciprofloxacin, 24 (36.36%) were sensitive to Benzylpenicillin, 42 (63.64%) were sensitive to clindamycin, 12 (18.18%) were sensitive to Levofloxacin, 30 (45.45%) were sensitive to Erythromycin and 42 (63.64%) were sensitive to Trimethoprim/Sulfamethoxazole.

All of the MRSA isolates were 50 (28.74%) resistant to Gentamicin, 170 (97.70%) were resistant to Ciprofloxacin, 98 (56.32%) were resistant to Clindamycin, 170 (97.70%) were resistant to Levofloxacin, 124 (71.26%) were resistant to Erythromycin, 96 (55.17%) to Trimethoprim/Sulfamethoxazole and 6 (3.45%) to Tigecycline. All the *Staphylococcus aureus* isolates were sensitive to Linezolid, Vancomycin, Tetracycline, and Teicoplanin.

Similarly, V. Vasuki & S. Ananthasankari et al. observed in their study that about 60-80% of MRSA isolates were resistant to Ciprofloxacin, Erythromycin, Gentamicin, and Tetracycline [13]. Similarly, Himani et al. has also reported that MRSA isolates were found to have multi-drug resistance. More than 70% of MRSA were resistant to Cotrimoxazole, Ciprofloxacin, Gentamicin, Erythromycin, and Tetracycline [18].

Likewise, Geeta S H et al. has reported 95.5% resistance to Ciprofloxacin & Ampicillin, 75% resistance to Gentamicin & 68% resistance to Erythromycin, 48% resistance to Cotrimoxazole & 52% to Clindamycin in MRSA isolates [12]. In their study, Sapkota J et al. observed that all MRSA strains were resistant to penicillin, 47.87% were resistant to Ciprofloxacin, 46.81% were resistant to Gentamicin, 42.55 were resistant to Cotrimoxazole, 34.04% were resistant to Tetracycline, 78.72% were resistant to Erythromycin and 78.72% were resistant to Clindamycin [17].

In a study from north India, the prevalence of MRSA was 30-70 percent and MRSA isolates were found to be more resistant to other antibiotics than MSSA [2, 4, 9, 10, 18].

In a study from south India, the prevalence of MRSA was 40-80 percent and MRSA isolates were found to be more resistant to other antibiotics than MSSA [8, 11, 13, 24].

In a study from other countries, the prevalence of MRSA was 30-80 percent [1, 6, 17, 21, 25].

Staphylococcus aureus, one of the oldest pathogens known is still one of the commonest causes of pyogenic infections in humans. Persistently high & increasing rates of MRSA among *Staphylococcus aureus* isolates have been observed in health care settings in many regions of India.

Worldwide, MRSA has emerged as a major cause of hospital-acquired infections. The possible predisposing factor is included that it increases the chance of emergence and spread of MRSA as a prolonged and repeated hospitalization, indiscriminate use of antibiotics, lack of awareness, intravenous drug abuse, and presence of indwelling medical devices. The prevalence of MRSA considerably varied among different studies with the highest rates of >50% in North and South India.

Conclusion:-

Staphylococcus aureus is an important cause of Hospital associated infections (HAI) and Community associated infections (CAI). Treating infections resulting from these microorganisms may be challenging due to the increasing range of *Staphylococcus aureus*, that are resistant to Beta-lactam antibiotics. Accurate designation of antimicrobial susceptibility pattern of the infecting microorganisms is a critically important element in making appropriate therapeutic decisions, due to multi-drug resistance in *Staphylococcus aureus* infections.

In our study total of 240 *Staphylococcus aureus* were isolated and the prevalence of MSSA was 27.5% and the prevalence of MRSA was 72.5% from various clinical samples. The maximum number of *Staphylococcus aureus* was isolated from the inpatient Department followed by ICU and outpatient Department.

Out of 66 (27.5%), MSSA isolates 42 (63.64%) were resistant to Ciprofloxacin, Benzylpenicillin, and Levofloxacin, followed by Erythromycin 30 (45.45%), Clindamycin 24 (36.36%), Trimethoprim/Sulfamethoxazole 24 (36.36%) and Gentamicin 6 (9.09%).

Out of 174 (72.5%), MRSA isolates all were resistant to Benzylpenicillin, followed by Ciprofloxacin 170 (97.70%), Levofloxacin 170 (97.70%), Erythromycin 124 (71.26%), Clindamycin 98 (56.32%), Trimethoprim/Sulfamethoxazole 96 (55.17%), Gentamicin 50 (28.74%), Rifampicin 15 (8.62%) and Tigecycline 6 (3.45%).

There was no resistance documented against Linezolid, Vancomycin, Tetracycline, and Teicoplanin. Resistance to antibiotic among the Methicillin-resistant *Staphylococcus aureus*, MRSA isolates was more than that in methicillin-sensitive *Staphylococcus aureus* (MSSA). All the 122 Clindamycin resistant isolates, 85 were inducible Clindamycin resistance.

The present study indicates the high prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) 174 (72.50%) and the low prevalence of Methicillin-sensitive *Staphylococcus aureus* (MSSA) 66 (27.50%) in our region.

Therefore, regular surveillance of hospital-associated infections is required for assessing the trends in the antibiotic resistance of pathogens for strengthening the control measure to prevent the spread and reduce the emergence of multidrug-resistant MRSA infections.

Irrational use of antibiotics, absence of antimicrobial stewardship program in hospitals, lack of surveillance and reporting system, failure to observe infection control practices like handwashing, and barrier nursing could be some reasons for this problem.

The antimicrobial sensitivity testing of MRSA and MSSA shows that MRSA is resistant to most antibiotics as compared to MSSA.

The most effective antibiotics for the treatment of MRSA were Linezolid, Vancomycin, Teicoplanin, and Tetracycline. These antibiotics should be kept reserved for the treatment of MRSA infection. Other options should be considered for the treatment of MSSA infection.

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