

Speciation and Antimicrobial Susceptibility of Coagulase Negative Staphylococci, Isolated from the Anterior Nares of Health Care Workers, in A Tertiary Care Hospital in South India, with Special Reference to Methicillin Resistance

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ABSTRACT

Introduction: Coagulase negative staphylococci (CoNS) are identified as emerging pathogens causing nosocomial infections, with significant morbidity and mortality. Speciation of CoNS helps in the understanding of their susceptibility patterns, the reservoirs and the epidemiology. CoNS are multidrug resistant and act as reservoirs for drug resistant genes. They are found in health care workers (HCWs), who act as reservoirs and help, in the spread of nosocomial infections. This study was undertaken to determine the occurrence, the species and susceptibility pattern of CoNS isolated from the anterior nares of HCWs working in our tertiary care hospital.

Material and Methods: Anterior nasal swabs were taken from a total of 310 HCWs. Speciation of CoNS was done by a practical scheme adopted from various references. Kirby Bauer disc diffusion method was performed as per CLSI guidelines. Statistical methodology: Percentage description of the data was given.

Results: The rate of isolation of CoNS was 55.8% (173/310). Among the 173 CoNS isolated in this study, 44%(76/173) were *S. haemolyticus*, 30% (52/173) were *S. warneri*, 14%(25/173) were *S. capitis*, 5% (8/173) were *S. simulans*, 4%(7/173) were *S. epidermidis*, 2%(3/173) were *S. schleiferi* and 1%(2/173) were *S. lugdenensis*. 16.18% of the isolates were MRCoNS. Doctors had the highest number of MRCoNS (10/28, 35.7%). Methicillin resistance was highest in *S. lugdenensis* (50%). Multidrug resistance was seen in the CoNS isolates. All isolates were sensitive to vancomycin.

Conclusion: This study reiterates the need to screen HCWs for CoNS and to adopt simple, economical and user friendly tests for speciation. The species and its susceptibility pattern help to eliminate reservoirs and prevent nosocomial infections.

Keywords: anterior nares, antibiotic susceptibility, Coagulase negative Staphylococci (CoNS), health care workers(HCWs), methicillin resistant Coagulase negative Staphylococci (MRCoNS), multidrug resistant CoNS, speciation of CoNS.

INTRODUCTION

Coagulase negative staphylococci (CoNS) are commonly found on human skin and several biotypes can be detected on a single individual.¹ In Microbiology laboratories, it is a common practice to identify coagulase negative Gram positive cocci as CoNS and the identification process stops there. It was also common to think that these organisms were not pathogenic and were dismissed as contaminants.² But in the last few years, various studies have demonstrated that CoNS are an emerging group of pathogens.²⁻⁴ They are associated with nosocomial infections.^{2,3,5} They are identified as the third commonest cause

of blood stream infections, which causes significant morbidity and mortality.⁵ Several reports of CoNS infections, involving indwelling foreign bodies, catheters and artificial devices are on the rise.³

It is important to identify CoNS up to the species level, as the epidemiology, the pathogenicity and drug resistance varies from species to species.⁵ Multidrug resistant strains have been reported from various studies.^{2,6} The challenge with CoNS is that, not only are they multi drug resistant, but they are known to act as reservoirs for drug resistant genes.⁷ The presence of such multidrug resistant strains and methicillin resistant strains in species of Staphylococcus, pose a challenge especially if they are found in the health care workers (HCWs). They act as reservoirs and help in nosocomial spread of infections. In addition, they cause problems for hospital infection control programmes in tertiary care hospitals.⁷

The data on the carriage rate of CoNS in HCWs is lacking.⁷ Hence, we undertook this study, to determine the occurrence, the species and susceptibility pattern of CoNS isolated from the anterior nares of HCWs working in our tertiary care hospital.

MATERIAL AND METHODS

This was a purposive sampling done on all health care workers (HCWs), for a duration of six months with an inclusion criteria being that all consenting HCWs working in our hospital to be included in the study. The exclusion criteria were: all non – consenting HCWs, HCWs who were on antibiotics, who had recent upper respiratory tract infection, who underwent recent nasal surgery and who had lesions in the nose, were to be excluded from the study.

We conducted a prospective study for 6 months duration among the health care workers (HCWs) in Raja Rajeswari Medical

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Group/species	Clumping factor	Tube coagulase	Ornithine decarboxylase	Urease	Novobiocin (5 µg)	Mannose	Species/Subspecies	Trehalose growth	Mannitol	Acetoin	Lactose	Anaerobic	Xylose
<i>S. epidermidis</i> group	-	-	+	+	S	+	<i>S. epidermidis</i> <i>S. caprae</i> <i>S. capitis</i> subsp. <i>ureolyticus</i>	- + -	- - +				
<i>S. haemolyticus</i> group	-	-	-	-	S	-	<i>S. haemolyticus</i> <i>S. auricularis</i> <i>S. caseolyticus</i>			+ - -	- - +		
<i>S. saprophyticus</i> group	-	-	-	+	R	-	<i>S. saprophyticus</i> subsp. <i>saprophyticus</i> <i>S. hominis</i> subsp. <i>novobiosepticus</i>	+					
<i>S. warneri</i> group	-	-	-	+	S	-	<i>S. warneri</i> <i>S. hominis</i> subsp. <i>hominis</i>					+	
<i>S. lugdunensis</i>	-	-	+	±	S	+							
<i>S. schleiferi</i> subsp. <i>schleiferi</i>	+	-	-	-	S	+							
<i>S. schleiferi</i> subsp. <i>coagulans</i>	-	+	-	+	S	+							
<i>S. simulans</i>	-	-	-	+	S	±							
<i>S. capitis</i> subsp. <i>capitis</i>	-	-	-	-	S	+							
<i>S. cohnii</i> subsp. <i>cohnii</i>	-	-	-	-	R	±	<i>S. xylosus</i>						+
<i>S. cohnii</i> group	-	-	-	+	R	+	<i>S. cohnii</i> subsp. <i>ureolyticum</i>						-

Table-1: Identification of CONS by simple scheme and additional tests

College and Hospital. Doctors, nurses, technicians and class IV workers were included in the study. The institutional ethical committee approved the study. All health care workers who consented to give samples were included in the study. Prior to enrollment in the study, written consent was obtained from the health care workers. Two pre-moistened swabs were used to swab the anterior nares of health care workers. One swab was inoculated on to Mannitol salt agar and the other swab was inoculated into BHI broth, after overnight incubation at 37°C it was subcultured on to blood agar plates. The CoNS gave red coloured colonies on Mannitol salt agar. These red coloured colonies were identified as CoNS based on colony morphology, Gram stain, catalase test, slide coagulase and tube coagulase test. To exclude Micrococci and Stomatococcus species, bacitracin susceptibility test was performed.⁵ To identify CoNS up to species level, we chose tests that were simple, user friendly and economical, from Kloos and Schleifer scheme, Mackie and Mc Cartney and Koneman et al.⁸⁻¹⁰ (Table-1).

The tests mentioned in Table-1 were used to identify the common species of CoNS, which are as follows: the *S. haemolyticus* group (*S. haemolyticus*, *S. auricularis* and *S. caseolyticus*), the *S. saprophyticus* group (*S. saprophyticus* subsp. *saprophyticus* and *S. hominis* subsp. *novobiosepticus*), the *S. epidermidis* group (i.e., *S. epidermidis*, *S. capitis* subsp. *ureolyticus* and *S. caprae*), the *S. warneri* group (*S. warneri* and *S. hominis* subsp. *hominis*), *S. lugdunensis*, *S. schleiferi* subsp. *schleiferi*, *S. capitis* subsp. *capitis*, *S. simulans* and *S. cohnii* subsp. *cohnii*, the *S. cohnii* group (*S. xylosus* and *S. cohnii* subsp. *ureolyticum*).⁴

This scheme involved a two-step procedure (Table-1), first step aimed to identify species group and combined slide and tube coagulase with novobiocin resistance, test for urease activity, ornithine decarboxylase and aerobic acid from mannose. If identification required additional tests, a maximum of two tests were selected from Table-1:

- i. Trehalose and mannitol for the *S. epidermidis* group
- ii. Acetoin production and lactose for the *S. haemolyticus* group
- iii. Trehalose for the *S. saprophyticus* group
- iv. Anaerobic thioglycollate broth for the *S. warneri* group
- v. Xylose for the *S. cohnii* group.

Standard protocol was followed to perform the above tests.^{4,8-10}

Antimicrobial susceptibility testing

Kirby Bauer disc diffusion method was performed for susceptibility testing. The following antibiotic discs were tested: amoxyclav (20 µg amoxicillin and 10 µg clavulanic acid), ciprofloxacin (5 µg), chloramphenicol (30 µg), clindamycin (2 µg), cotrimoxazole (1.25/23.75 µg), cefoxitin (30 µg), doxycycline (30 µg), erythromycin (15 µg), gentamicin (10 µg), linezolid (30 µg), oxacillin (1 µg) penicillin (10 units) and vancomycin (30 µg).

Methicillin resistance was screened using disc diffusion method. Four to five colonies from overnight growth were inoculated into peptone water. This was incubated at 35°C till it matched a turbidity standard of 0.5 McFarland. Cefoxitin (30 µg, Hi-Media, Mumbai, India) was used to identify methicillin resistant coagulase negative staphylococci (MRCoNS). Zone size of ≤ 24mm or less is considered as resistant to cefoxitin, for all CoNS, except for *S. lugdenensis*, for which zone diameter ≤ 21mm is considered resistant to cefoxitin.^{5,7,11} Quality control was done using ATCC strain *Staphylococcus aureus* 25923.

STATISTICAL ANALYSIS

SPSS version 20 software was used for the statistical analysis. Descriptive analysis was given in terms of percentages to infer data.

RESULTS

Out of the 310 HCWs included in the study, 94(30.3%) were males and 216 (69.7%) were females. The anterior nares of 310 HCWs were swabbed. Of which, 105(33.8%) were attenders, 78 (25.2%) were doctors, 75 (24.2%) were technicians and 52 (16.8%) were nurses. Of the 310 HCWs who were included in this study, 60 % (186/310) were aware of health care associated infections. The various isolates that were isolated in this study are as follows: 55.8% (173/310) CONS, 25.5 % (79/310) Micrococcus species and 9% (28/310) *S.aureus*. 9.7 % (30/310) of the nasal swabs yielded no growth. Among the 173 CoNS isolated in this study, 44% (76/173) were *S. haemolyticus*, 30% (52/173) were *S. warneri*, 14%(25/173) were *S. capitis*, 5% (8/173)were *S. simulans*, 4% (7/173) were *S. epidermidis*, 2% (3/173) were *S. schleiferi* and 1% (2/173) were *S. lugdenensis* (Figure-1).

67.94% (53/78) of the doctors, 55.23% (58/105) of the attenders, 50% (26/52) of the nurses and 48% (36/75)of technicians were positive for CoNS (Table-2).The HCWs who tested positive for CoNS, were distributed in our hospital as follows: 19.7% (34/173) of CoNS were isolated from Pharmacy, 11.6% (20/173) of CoNS were isolated from Laboratory, 11% (19/173) of CoNS were isolated from OBG ward, 9.8% (17/173) from Surgery ward, 9.2% (16/173) from Orthopaedics ward, 9.2% (16/173) from OT, 8.7% (15/173) from Paediatrics ward, 8.1% (14/173) from Medicine ward, 6.9% (12/173) from ICU, 2.3% (4/173) from ENT, 1.7% (3/173) from Psychiatry, 1.2% (2/173)from

Radiology and 0.6%(1/173)from Ophthalmology (Figure-2). *S. haemolyticus* showed resistance to the following antibiotics- 6.6% (5/76) resistance was seen to amoxicillin clavulanic acid, 5.3% (4/76) to chloramphenicol, 14.5% (11/76) to cefoxitin, 7.9% (6/76) each to ciprofloxacin and doxycycline, 32.9% (25/76) to clindamycin, 17.1% (13/76) to cotrimoxazole, 57.9% (44/76) to erythromycin, 3.9% (3/76) to gentamicin, 1.3% (1/76) to linezolid, 11.8% (9/76) to oxacillin and 61.8% (47/76) to penicillin. No resistance was seen to vancomycin (Table-3). *S. warneri* showed 7.7% (4/52) resistance to each of the following antibiotics: chloramphenicol, ciprofloxacin, doxycycline, gentamicin and oxacillin. It showed 11.5% (6/52) resistance to amoxicillin clavulanic acid, 13.5% (7/52) to cefoxitin, 26.9% (14/52)to clindamycin, 17.3% (9/52) to cotrimoxazole,

HCW group	Number screened	No. of CoNS isolated (Percentage %)	No. of MR-CoNS isolated (Percentage %)
Attenders	105	58(55.23)	9(32.2)
Doctors	78	53(67.94)	10(35.7)
Technicians	75	36(48)	6(21.4)
Nurses	52	26(50)	3(10.7)
Total	310	173(55.8)	28(100)

Table-2: Isolation of CoNS and MRCoNS in the various groups sampled

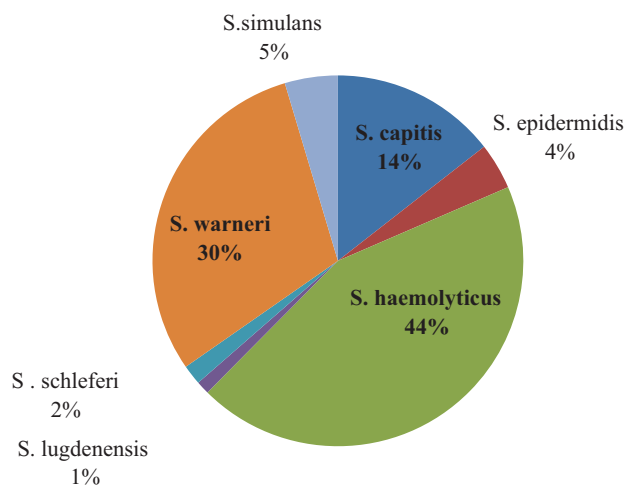


Figure-1: The various species of Coagulase negative Staphylococci isolated

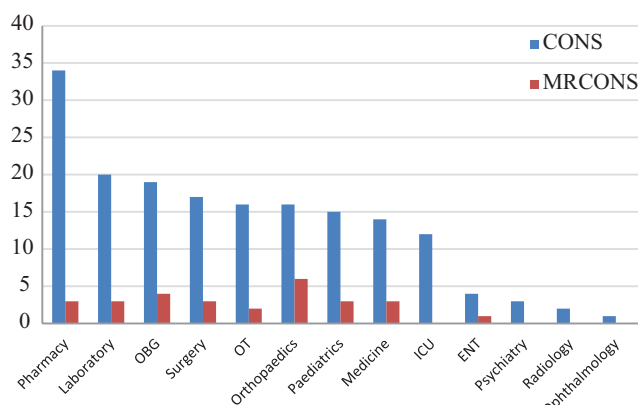


Figure-2: Distribution of HCWs who tested positive for CoNS and MRCoNS in different areas of the hospital.

	Amoxicillin clavulanic acid		Cefoxitin		Chloramphenicol		Ciprofloxacin		Clindamycin		Cotrimoxazole		Doxycycline		Erythromycin		Gentamicin		Linezolid		Oxacillin		Penicillin		Vancomycin	
	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
<i>S. haemolyticus</i> (76)	5	71	11	65	4	72	6	70	25	51	13	63	6	70	44	32	3	73	1	75	9	67	47	29	0	76
<i>S. warneri</i> (52)	6	46	7	45	4	48	4	48	14	38	9	43	4	48	27	25	4	48	1	51	4	48	25	27	0	52
<i>S. capitis</i> (25)	2	23	6	19	0	25	2	23	9	16	4	21	2	23	9	16	1	24	1	24	5	20	11	14	0	25
<i>S. simulans</i> (8)	1	7	1	7	1	7	1	7	1	7	2	6	1	7	3	5	1	7	1	7	1	7	5	3	0	8
<i>S. epidermidis</i> (7)	0	7	1	6	0	7	0	7	1	6	0	7	0	7	0	7	0	7	0	7	1	6	2	5	0	7
<i>S. schleiferi</i> (3)	1	2	1	2	0	3	0	3	0	3	0	3	1	2	1	2	0	3	0	3	1	2	2	1	0	3
<i>S. lugdenensis</i> (2)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	0	2	1	1	1	1	0	2
Total	16	157	28	145	10	163	14	159	51	122	29	144	15	158	85	88	9	164	4	169	22	151	93	80	0	173
Percentage (%)	9.25	90.75	16.18	83.82	5.78	94.22	8.09	91.91	29.48	70.52	16.76	83.24	8.67	91.33	49.13	50.87	5.20	94.80	2.31	97.69	12.72	87.28	53.76	46.24	0.00	100.00

Table-3: Resistance pattern of the various species of CoNS.

51.9% (27/52) erythromycin, 1.9% (1/52) linezolid and 48.1% (25/52) to penicillin. No resistance was seen to vancomycin (Table-3).

S. capitis showed resistance to the following antibiotics - 8% (2/25) resistance was seen to amoxicillin clavulanic acid, 24% (6/25) to cefoxitin, 8% (2/25) to ciprofloxacin, 36% (9/25) to clindamycin, 16% (4/25) to cotrimoxazole, 8% (2/25) to doxycycline, 36% (9/25) to erythromycin, 4% (1/25) to gentamicin, 4% (1/25) to linezolid, 20% (5/25) to oxacillin, 44% (11/25) to penicillin. No resistance was seen to chloramphenicol and vancomycin (Table-3).

S. simulans showed 12.5% (1/8) resistance to each of the following antibiotics: Amoxicillin clavulanic acid, cefoxitin, chloramphenicol, ciprofloxacin, clindamycin, doxycycline, gentamicin, linezolid and oxacillin. It showed 25% (2/8) resistance to cotrimoxazole, 37.5% (3/8) to erythromycin and 62.5% (5/8) to penicillin. No resistance was seen to vancomycin (Table-3).

S. epidermidis showed 14.3% (1/7) resistance to clindamycin, cefoxitin and oxacillin, 28.57% (2/7) resistance to penicillin. No resistance was seen to amoxicillin - clavulanic acid, chloramphenicol, ciprofloxacin, cotrimoxazole, doxycycline, erythromycin, gentamicin, linezolid and vancomycin (Table-3).

S. schleiferi showed 33.3% (1/3) resistance each, to amoxicillin clavulanic acid, cefoxitin, doxycycline, erythromycin and oxacillin. 66.6% (2/3) resistance was seen to penicillin. No resistance was seen to chloramphenicol, ciprofloxacin, clindamycin, cotrimoxazole, gentamicin, linezolid, and vancomycin (Table-3).

In *S. lugdenensis*, 50% (1/2) resistance was seen to each of the following: amoxicillin clavulanic acid, cefoxitin, chloramphenicol, ciprofloxacin, clindamycin, cotrimoxazole, doxycycline, erythromycin, oxacillin and penicillin. No resistance was seen to gentamicin, linezolid and vancomycin (Table-3).

Of the 28 MRCoNS isolated, 10 (35.7%) were from doctors, 9 (32.2%) were from attenders, 6 (21.4%) were from technicians and 3 (10.7%) were from nurses (Table-3). The distribution of the HCWs in whom MRCONS were isolated is as follows: 21.4% (6/28) of MRCONS were isolated from Orthopaedics, 14.3% (4/28) of

MRCONS were isolated from OBG ward, 10.7% each (3/28) of MRCONS were isolated from Medicine ward, Surgery ward, Paediatrics, Laboratory and Pharmacy respectively. 7.2% (2/28) of MRCONS from OT and 3.6% (1/28) from ENT (Figure-2)

DISCUSSION

In recent years CoNS have been identified as an important nosocomial pathogen. It is important that we identify them up to the species level, as it helps in identifying the reservoir, studying the distribution of CoNS implicated in the causation of nosocomial infections and in determining the etiological agent.⁴ For species identification, tests that were simple, user friendly and economical were chosen (Table-1).

A total of 310 HCWs were included in the study. 69.7% of the HCWs included in this study were females, as more number of females were included in the study as compared to males. Of the 310 HCWs who were sampled, 173 HCWs yielded CoNS. The rate of isolation of CoNS was 55.8% (173/310). This correlates with the study conducted by Narayani et al. who reported a nasal carrier rate of 62% CoNS.¹² The percentage of isolation of CoNS was highest in the doctors (67.94%), followed by attenders (55.23%), nurses (50%) and technicians (48%). 57% of the HCWs, who participated in the study, were aware about health care associated infections. For the remaining 43% of HCWs, our hospital conducts several training and informative programmes to improve their knowledge about health care associated infections.

The various species of CoNS that were isolated are as follows: 44% (76/173) were *S. haemolyticus*, 30% (52/173) were *S. warneri*, 14% (25/173) *S. capitis*, 5% (8/173) were *S. simulans*, 4% (7/173) were *S. epidermidis*, 2% (3/173) were *S. schleiferi* and 1% (2/173) were *S. lugdenensis*. Studies conducted by, Mohan U (82.29%), Goyal R (41%), Shobha KL (49.23%) report *S. epidermidis* as their predominant isolate.¹⁻³ In our study, *S. haemolyticus* (44%) was the predominant isolate, followed by *S. warneri* (30%) and *S. capitis* (5%). *S. epidermidis* was the fifth highest isolate (4%) in this study. Highest number of CoNS was isolated from the anterior nares of HCWs working in the Pharmacy (19.7%), followed by HCWs

working in the laboratory (11.6%) and in the OBG department (11%) (Figure-2). However, the highest number of MRCoNS were isolated from the HCWs working in Orthopaedics (6/28, 21.4%), followed by those working in OBG (4/28, 14.3%) (Figure-2). Therefore, it is important to include all the hospital staff, working in all areas of the hospital for surveillance studies.

In a study conducted by KL Shobha 22.22% of MRCoNS were reported from anterior nares.⁷ In our study, 16.18% (28/173) of the isolates were MRCoNS. Anterior nares of HCWs Doctors had the highest number of MRCoNS (10/28, 35.7%). These findings reflect that doctors are important in the chain of transmission of nosocomial infections and have to upgrade their compliance with hospital infection control programmes.

With regards to susceptibility testing, multidrug resistance was seen in many isolates. This correlates with the studies conducted by Mohan U, Goel MM and Pathak J.^{1,13,14} Maximum resistance was seen towards drugs like penicillin (53.76%), erythromycin (49.13%), clindamycin (29.48%), cotrimoxazole (16.76%), cefoxitin (16.18%) and amoxiclav (9.25%). *S. lugdenensis* was the only species which showed resistance to almost all the antibiotics on the testing panel except for gentamicin, linezolid and vancomycin. Methicillin resistance was observed in all the species of CoNS isolated in this study. The percentages are as follows: 50% methicillin resistance in *S. lugdenensis*, 33.3% in *S. schleiferi*, 24.5% in *S. capitis*, 14.5% in *S. haemolyticus*, 14.3% in *S. epidermidis*, 13.57% in *S. warneri* and 12.5% in *S. simulans*.

There are reports of emerging vancomycin resistance among Methicillin resistant CoNS.¹⁶ In our study, all isolates were sensitive to vancomycin. The variability in antibiotic susceptibility pattern is because, we have different species of CoNS as our predominant isolates, there is a geographical variation and, differences in the antibiotic panel used in every hospital.

Today there are several molecular tests, which detect genes like the *ica* gene (intercellular adhesion – operon – *ica* ADBC), *atlE* gene (encodes for the vitronectin – binding cell surface protein involved in primary attachment) and the *mecA* gene (controls the synthesis of PBP2a).³ Other tests like plasmid analysis, tests for slime production and adherence help in the better understanding of pathogenesis, diagnosis and epidemiology of CoNS.¹⁵ These tests may not be economically viable for all hospitals to carry out.

CONCLUSION

Therefore, this study, reiterates the need to speciate the CoNS with an easy, user friendly and economical tests. It is important to speciate the CoNS as this study has proved that the species reported in other hospitals (*S. epidermidis*) may not coincide with the species isolated in our hospital (*S. haemolyticus*). The study also presses the need to screen the HCWs for carriage of CoNS on a regular basis, as these HCWs act as reservoirs for CoNS. CoNS are identified as emerging pathogens and are known for multidrug resistance. CoNS could be potential roadblocks to hospital infection control programmes. The species and its corresponding sensitivity pattern have to be kept in mind so as to eliminate reservoirs and prevent the spread of nosocomial infections.

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