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RESEARCH ARTICLE

SPECIATION AND RESISTOTYPING OF COAGULASE NEGATIVE STAPHYLOCOCCI FROM CLINICAL SAMPLES: A CROSS-SECTIONAL STUDY AT TERTIARY CARE HOSPITAL FROM CENTRAL INDIA

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ABSTRACT:

Background: Negative coagulase Consisting of native bacteria found on human skin and mucous membranes, staphylococci (CONS). They were hardly ever known to produce serious infections and were long thought to be non-pathogenic. However, CONS have emerged as the primary cause of nosocomial blood stream infections, accounting for 9% of all nosocomial infections, as a result of a combination of rising intravascular device use and a rise in the number of hospitalized immunocompromised patients. The organisms causing these illnesses are resistant to many drugs, making treatment challenging.

Objective: Coagulase-negative Staphylococci are isolated, identified, and speciated from a variety of clinical specimens, and their antibiotic susceptibility pattern is examined.

Material and Methods: 200 strains of CONS were identified from exudates, urine, and blood of clinically confirmed cases using established identification techniques, out of 2560 samples obtained for this investigation. Using the Kirby-Bauer disc diffusion method, the isolated strain's antibiotic susceptibility was evaluated.

Results: S. epidermidis (59%), S. saprophyticus (29%), S. haemolyticus (9%), S. xylosus (2%) and S. capitis (1%), were the most frequently isolated CONS species. Maximum amounts of S. saprophyticus (74.19%) and S. epidermidis (72%), respectively, were isolated from exudates and urine. According to the tests for antibiotic susceptibility, vancomycin was the most effective medication, followed by ciprofloxacin. CONS with methicillin resistance were 38%.

Conclusion: S. epidermidis was the most often isolated species, indicating that it is a significant pathogen and not only a commensal. The fact that S. saprophyticus was the most often isolated urine pathogen suggests that it is a prevalent urinary pathogen. Methicillin resistance is a major issue that requires careful consideration and in-depth research, and the antibiotic resistance pattern in CONS poses a serious concern to practitioners.

Key Words: Antibiotic susceptibility, clinical isolates, coagulase negative staphylococci, identification.

INTRODUCTION:

Negative coagulase Staphylococci (CONS), which were once written off as pollutants, are becoming significant potential pathogens due to the rise in patients who are really sick and the growing use of implants in hospitals[1]. Though more than 30 species of CONS are known to exist, only a small number of them are frequently linked to infections in humans. Often, strains are resistant to many drugs. Antibiotic-resistant strains can originate from the skin of patients and healthcare personnel, medical equipment, personnel clothing, and environmental surfaces[2-4]. In patients with indwelling medical devices, such as pacemakers, valvular prostheses, artificial heart valves, central and peripheral venous catheters, and orthopaedic prostheses, CONS is one of the primary causative agents of bacteraemia. Recently, many CONS species have been identified[5].

The species that is most commonly isolated from infections is Staphylococcus epidermidis. It has been identified as the causative agent in infections of the skin, meninges, urogenital tract, respiratory tract, and wounds. In women, coliforms are the most frequent cause of acute urethral syndrome, followed by S. saprophyticus. A precise determination of the host-pathogen relationship of CONS is made possible by reliable species identification, which is extremely desirable due to the growing clinical significance of

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CONS[6]. Public health is concerned about the rise in antibiotic resistance observed globally. When antibiotics are used appropriately, numerous adverse effects are minimized, needless costs are decreased, and resistance to beneficial and life-saving antibiotics is slowed down. To use antibiotics sensibly, one must be aware of the susceptibility/resistance pattern of CONS[7].

There is a dearth of information on CONS that cause infections in underdeveloped nations. According to a study done at Kasturba Medical College Hospital in Manipal, 13.84% of all CONS isolates had MRCONS. A different survey conducted in India found that 62.7% of clinical isolates have MRCONS[8]. Given the significance of CONS as a nosocomial infection causative agent, the current investigation was conducted to examine the species distribution and susceptibility to antibiotics of CONS isolated from various clinical specimens.

MATERIALS AND METHODS

After receiving approval from the Institutional Ethical Committee, the current study was carried out. Over the course of 18 months, 200 strains of CONS were identified and examined. Patients with clinical diagnoses of respiratory tract infections, septicemia, peritonitis, cervicitis, conjunctivitis, pyogenic infections (burn wound infection, post-operative wound infection, osteomyelitis, pyoderma, chronic suppurative otitis media, corneal ulcer, diabetic ulcer) and pyogenic infections were the patients from which the samples were obtained. The study only included isolates that were cultivated repeatedly in pure culture. Colony morphology, Gram stain, the Catalase test, and the Coagulase test (Slide and Tube Coagulase) were used to specifically identify CONS. Using Bacitracin susceptibility testing, Micrococci and Stomatococcus species were ruled out.

The Novobiocin sensitivity test (5 µg), the urease and phosphatase tests, and the fermentation of glucose, sucrose, mannitol, maltose, and xylose are all performed in order to determine further speciation. The Kirby- Bauer disc diffusion method was used to assess the isolated strain's antibiotic susceptibility in accordance with Clinical and Laboratory Standards Institute (CLSI) recommendations. Ten different antibiotics were evaluated in a panel: cephalexin (Cn), cefotaxime (Ctx), gentamicin (G), erythromycin (E), vancomycin (Va), ampicillin (Amp), cefotaxime (Amc), and co-trimoxazole (Cot). The method for identifying methicillin-resistant CONS (MRCONS) was cefoxitin (30µg).

RESULTS

Table 1: Age and sex wise distribution of CONS

Age group	Male		Female		Total	
	No.	%	No.	%	No.	%
< 1 yr	2	2.6	4	3.2	6	3
1 - 14	6	7.9	8	6.5	14	7
15 – 45	48	63.2	96	77.4	144	72
46 - 60	4	5.3	12	9.7	16	8
> 60	16	21.1	4	3.2	20	10
Total	76	38	124	62	200	100

According to the above statistics, of the 200 CONS that were isolated, more isolates came from female patients (62%) than from male patients (38%). The age group most frequently afflicted was 15–45 years old (72%), then > 60 years old (10%), 46–60 years old (8%), 1–14 years old (7%), and less than one year (3%). The age range of 15-45 years old had the highest percentage of both males and females (77.42%) and (63.15%), respectively. The majority of the 200 CONS that were isolated came from exudates (50%), urine (31%), and blood (19%). Eighty exudates from pus, six from ear swabs, six from vaginal swabs, four from pleural fluid, and four from catheter tips were isolated.

S. epidermidis accounted for the majority of CONS species that were isolated (59%), with S. saprophyticus (29%), S. haemolyticus (9%), S. xylosus (2%) and S. capitis (1%). Of the 100 exudate samples, S. epidermidis accounted for 72% of the isolates, followed by S. haemolyticus (14%), S. saprophyticus (12%), and S. xylosus 2%. S. saprophyticus accounted for 74.19 percent of the 62 urine samples, followed by S. haemolyticus (16.12%), S. xylosus, S. epidermidis, and S. capitis (3.22%) each. Out of 38 blood samples,

S. haemolyticus made up 5.27 percent and S. epidermidis, 94.73%. Of the 200 CONS that were isolated, 62% were sensitive to methicillin and 38% were resistant.

Table 2: Antibiogram of CONS

Antibiotic	Resistant		Sensitive	
	No.	%	No.	%
Ampicillin	122	62	76	38
Amoxy-clav	48	24	142	76
Erythromycin	108	54	92	46
Vancomycin	0	0	200	100
Gentamicin	40	20	160	80
Tetracycline	64	32	136	68
Ciprofloxacin	16	8	184	92
Cephalexin	106	53	92	47
Cefotaxime	42	21	158	79
Co-trimoxazole	84	42	116	58

The table above demonstrates that every strain of CONS exhibited vancomycin sensitivity. They did, however, exhibit low resistance to ciprofloxacin 8% and resistance to ampicillin (62%), erythromycin (54%), cephalexin (53%), and co-trimoxazole (42%).

DISCUSSION

The primary cause of nosocomial infections has been CONS. Multidrug resistant MRCONS are becoming more common, which is causing hospitals and communities to struggle. The development of biofilms and the prevalence of antibiotic resistance make treatment particularly challenging[9,10]. One of the main problems facing clinical microbiologists is differentiating between contaminant strains and clinically relevant pathogenic pathogens. Many isolates have infections that are difficult to treat and sometimes even lethal because they are resistant to many antibiotics. Now that CONS have been isolated from clinical specimens more frequently, each one needs to be assessed separately as a possible genuine pathogen. Out of the 200 CONS that were isolated in this study, the majority (62%) came from females. This finding was consistent with a study by Namrata Kumari et al. (2001), which found that the majority of CONS isolates came from females (54.10%). In the current investigation, the majority of CONS species were isolated from the 15–45 year age range in both girls (77.42%) and males (63.15%). (See Table No. 1) These results are consistent with a 2012 study conducted in Iran, which found that the majority of CONS isolates in both sexes are between the ages of 30 and 44. However, the bulk of isolates were found in males 45 years of age or older, according to several investigations[11].

The high number of UTI cases (31%) during this time period is likely the reason why females in the 15–45 age range in the current study have a preponderance of CONS isolates from them. It is commonly known that women in the reproductive age range experience UTIs more frequently. The bulk of the isolates in this investigation came from pyogenic skin lesions such abscesses and wound swabs. The majority of the 200 CONS that were isolated came from exudates (50%) and urine (31%), followed by blood (19%). This is consistent with a different study that Surekha Y. Asangi et al. (2011) conducted in south India[12-14]. Nonetheless, the bulk of isolates from urine have been reported by Sheikh and Mehdinejad (2012) and Mohan U et al. (2002). The majority of CONS species isolated in this investigation were S. epidermidis (59%), which was followed by S. haemolyticus (9%), S. xylosus (2%) and S. capitis (1%). These results are consistent with another study by Surekha Y Asangi et al. (2011) that was carried out in south India, where S. haemolyticus 19 (19.7%) and S. saprophyticus 26 (27.1%) were the most common isolates, followed by S. epidermidis 43 (44.8%). Nonetheless, additional research has identified S. hemolyticus (18%) as the second most prevalent species. In this investigation, the prevalence of methicillin resistance was 38%. This is consistent with research conducted in 2010 at Government Medical College and Hospital, Anantapur (39.4%). Numerous other people have noted a far greater frequency of MRCONS[15].

The fact that our specimens were from hospitalized patients with no antibiotic policy in place may account for the comparatively higher rate of MRCONS isolates in our investigation. Inadequate implementation of antibiotic policy leads to widespread indiscriminate antibiotic use. Furthermore, the hospital's hygienic conditions and surroundings were inadequate. The spread of infectious pathogens is facilitated by patient and attendant overcrowding. Hospital acquired infections should be rather common in various wards under such circumstances. Consequently, infections with multidrug-resistant bacteria occur throughout hospital stays for patients. In our investigation, not a single isolate displayed vancomycin resistance. Others have observed a decreased vulnerability to vancomycin, though.

CONCLUSION

While the pathogenic role of CONS is now well recognized, the many species' clinical importance is still being determined. Until the clinical importance of any organism is established, we shouldn't rule it out. Nonaureus isolates are simply reported as CONS without speciation in the hospital microbiology lab. Due to the growing pathogenecity of these organisms, CONS should be detected using a straightforward, dependable, and ideally low-cost technique down to the species level. Methicillin resistance is a major issue that requires careful consideration and in-depth research, and the antibiotic resistance pattern in CONS poses a serious concern to practitioners. The question of whether some species are more resistant to antibiotics than others may be answered in the future by analyzing patterns of antibiotic resistance particular to individual species.

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