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COAGULASE NEGATIVE STAPHYLOCOCCI....AN EMERGING PATHOGEN IN HEALTH CARE FACILITIES.

Microbiology.

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ABSTRACT

Coagulase negative staphylococci (CoNS) have always been considered as contaminants in routine culture. But their potential to emerge as nosocomial pathogens is rising. The aim of our study was to identify, speciate, analyze the antibiogram and pathogenic potential of CoNS. 50 samples from suspected cases were cultured and CoNS identified to species level and their antibiogram performed. Significant CoNS isolates were from < 20yrs of age (26%) and most were from males (87.5%). Blood cultures yielded (52%) followed by pus (24 %). *S.epidermidis* was the most common species (36 %) followed by S. hemolyticus (20%). Vancomycin (98%) and Amikacin (92 %) were useful in treatment of resistant cases. Keeping in mind the pathogenic potential of CoNS, there is a need to identify, speciate, treat and prevent these infections in health care facilities.

KEYWORDS

Coagulase negative staphylococcus, Emerging CoNS infection, MRCoNS.

Introduction:

The last two decades have seen the potential of CoNS as a pathogen causing nosocomial and community acquired urinary tract infections.[1] An awareness of speciation has led researchers and clinicians alike to rethink the importance of CoNS.[2] A study by Usha MG et al found that *S. epidermidis* was the most frequently isolated CoNS followed by *S. haemolyticus. S. lugdunensis, S. hominis, S. saprophyticus, S. capitis, S. caprae, S. xylosus, S. cohnii and S. warneri.*[2] *S. saprophyticus* primarily cause acute urinary tract infections (UTI) in young healthy, sexually active women [3,1,4] and is also the second most common organism responsible for uncomplicated cystitis in young women after *Escherichia coli*.[5,6,4] CoNS also causes native valve endocarditis, infections of semi-permanent venous access devices, surgical wounds, prosthesis, CSF shunts, peritoneal dialysis and the eye.[7] Some predisposing host factors for CoNS infections include, immunocompromised states, chronic illnesses, presence of foreign body and indwelling prosthetic medical devices.[8] Their virulence factors range from extracellular toxins, enzymes and slime, promoting infection on the surfaces of implanted foreign bodies.[9] Other virulence factors include lipases, proteases and exoenzymes, that helps bacterial adherence and spread within the body.[10]

Several commercial kits and automated instruments can identify CoNS accurately but are still out of reach of most of the labs in developing countries.[11] The rapid and accurate identification of CoNS species has acquired importance in the last few years with commercial kits like Staph-Zym and API-Staph also available. However even though these system displays good sensitivity, there are chances of mis-identification and un-identification of CoNS isolates.[12] Thus it may be apt to depend largely on relatively convenient, reliable and inexpensive identification methods that can be adopted universally in all diagnostic labs both big and small.[11]

Wide spread use of broad-spectrum antibiotics for treatment of CoNS infection has led to the development of antibiotic resistance.[13] Needless to say methicillin-resistant CoNS (MRCoNS) are cross-resistant to all other ß-lactam antibiotics.[14] The antibiogram of CoNS can be done by agar dilution, oxacillin agar screen method, disk diffusion method and also by automated methods like Microscan Pos Combo panel Type 6. The gold standard for detection of resistance in CoNS to methicillin is detection of *mecA* gene by PCR. However this methodology cannot be done routinely in most laboratories.[15] Routinely most labs find the Kirby-Bauer disc diffusion method using a select panel of antibiotics, which includes ampicillin (A), amoxyclav (Amc), ceftriaxone (Ci), cotrimoxazole (Co), cefotaxime (Ce), gentamicin (G), amikacin (Ak) and vancomycin (Va) and a differential disc Cefoxitin (Cn-30 µg) to identify MRCONS more economical and convenient.[2] The variations in susceptibility pattern of CoNS do exist as described by U Mohan et al which may be due to different antibiotic protocols in various hospitals.[16] Thus the bottom line in tackling the menace of this emerging CoNS isolate lies in its rapid identification, accurate speciation and an elaborate antibiogram.

Material and methods:

This study was conducted at a tertiary care centre in Mangalore from 1st December 2014 to 30th November 2015. A total of 50 clinical samples that isolated CoNS in pure culture from various sites after ensuring that commensal flora are not included were processed for culture, sensitivity and identification in the microbiology laboratory. All the samples of patients other than infections with CoNS were excluded from the study. The clinical specimens that grew *Staphylococci* were identified by colony morphology, Gram stain, catalase and coagulase test. Once its identity as CoNS by slide and tube coagulase were established it was further subjected to biochemical tests as selected from the identification protocol described by Kloos and Schleifer et al,[17] which included; anaerobic growth in thioglycollate, alkaline phosphatase, arginine dihydrolase, ornithine decarboxylase, nitrate reduction, urease, and sugar fermentation of maltose, fructose, sucrose, lactose, mannitol, trehalose and xylose. Further differential disc testing for novobiocin, polymixin B and bacitracin sensitivity were also performed. Cefoxitin (30µg) was used to identify methicillin-resistant coagulase negative staphylococci (MRCoNS). Antibiotic sensitivity testing for ampicillin, amoxicillin, cefazolin, cefuroxime, co-trimoxozole, gentamicin, amikacin, ciprofloxacin, azithromycin and vancomycin were done as per CLSI guidelines. CoNS ATCC strain 12228 was used as controls. The statistical analysis was done by percentage method.

Results:

A total of 50 CoNS were isolated from various clinical specimens. The sex wise distribution showed a preponderance of CoNS infections in males 34 (68%). The age group < 20 yrs had the maximum number of isolates 13 (26%) followed by ages 51-60 with 9 (18%) and 41-50 with 8 (16%). The maximum number of CoNS isolated from blood samples were 26 (52%), pus sample 12 (24%) followed by wound swab 7 (14%). *S.epidermidis* 18 (36%) was the predominant isolate from most clinical specimens followed by *S.haemolyticus* 10 (20%) and then by *S.saprophyticus* 7 (14%). Out of 50 CoNS isolates, 10 (20%) were from Intensive Care Units. Majority of the CoNS species were sensitive to Vancomycin (98%) followed by Amikacin (92%) and Gentamicin (76%). Least sensitivity was shown towards Ampicillin (20 %), followed by Azithromycin (36%), Cefuroxime (42%) and resistance to Cefoxitin (30µg) ie. MRCoNS strains were 29 (58%).

Discussion:

To know the pathogenic role of each individual CoNS species and their clinical significance should be a top priority. The age group of < 20 yrs were more vulnerable to CoNS in our study (26%) whereas Sureka et al reported (39.5%) cases in > 40 years of age.[18] Her study showed a preponderance in males (65.6%) than females (34.4%) which was similar to our study. Among the males, majority of the CoNS (47.6%) were isolated in the > 40 years age group and in

females (27.2%) between 30-40 years similar to a study by Larry M. Baddour, and David L. Et al who reported (54.2%).[18] The slime of CoNS forms a biofilm colonizing catheters from where it multiplies and disseminates and it can function as a penetration barrier to antibiotics thus promoting drug resistance.[2] In our study slime may have contributed to some of the blood stream infections encountered. Likewise *S.saprophyticus* has urease enzyme and can adhere to uroepithelial cells thus producing UTI's [19,20] Our *S.saprophyticus* isolate however was from an indwelling central venous line.

Sample selection from the focus of infection without collecting the surrounding normal flora is the key to better diagnosis.[21] Of the 13 patients in the < 20 yrs category, 10 samples grew *S. epidermidis* from both central and peripheral lines of patients in medical intensive care. Chang Deo et.al [20] additionally isolated *S. haemolyticus. S.capitis ssp capitis, S.capitis ssp ureolyticus* and *S.cohnii ssp urealyticum* were some of the rare isolates in our study from blood culture.

S.epidermidis is an emerging pathogen in immunocompromised patients causing septicemia in leukemia and lymphoma patients.[22] *S. epidermidis, S. haemolyticus, S. lugdunensis* and *S. hominis* commonly cause cutaneous lesions.[23] Most of our isolates from pus samples were *S. epidermidis* followed by *S.xylosus, S.warneri* and *S.hominis*.

Similar biochemical traits of CoNS species, pose difficulties in correct identification thus PCR is a suitable alternative.[24] It is clear from our data and other studies from India and abroad by Chang Deo et al[7,20] that *S.epidermidis, S.haemolyticus and S.saprophyticus* are the most common isolates. But the role of other species like *S. hominis, S.warneri, S.simulans, S. lugdunensis, S. schleiferi, S. saccharolyticus and S. cohnii* in humans cannot be ignored.[25] We had a difficulty in identifying methicillin resistant *S.pasteuri* from blood culture in a case of septicemia and had to depend on Phoenix Identification system. Many of our CoNS isolates were resistant to commonly used antimicrobials similar to a study by Mitt etal.[26] and Chang Deo et al.[20] Multidrug resistance in *S. epidermidis* has a strong association between prolonged hospitalization especially in surgical ICU and parenteral hyperalimentation.[26] Our CoNS antibiogram showed resistance to ampicillin and azithromycin. *S.xylosus* was found resistant to ciprofloxacin and azithromycin (33.3%). *S.capitis ssp capitis* was the only isolate that showed resistance to vancomycin. Most of the other species like *S.capitis ssp ureolyticus, S.cohnii ssp cohnii* and *S.cohnii ssp urealyticum* were found to be sensitive to most antimicrobials. 92% of our CoNS strains were sensitive to Amikacin and this was in agreement with the study conducted by Goyal R et al.[7] The glycopeptide vancomycin a reserve drug in MRCoNS isolates was susceptible in 98% of our isolates as in studies by Usha M.G et al.[2] Accuracy and promptness in the detection of methicillin resistance is very much important in ensuring correct antibiotic treatment in the infected patients so that the control of MRCoNS can be achieved in hospital environments. High incidence of MRCoNS was reported by Seetha et al., (82.77%), Singhal R et al., (72.3%) and Jain, Agarwal and Bansal (66.0%) and the incidence range of MRCoNS varies from 14.6% to 38.0% as was also seen in our study.

Conclusion:

CoNS has a truly pathogenic potential than was earlier thought. The aim of every health care facility must be to accurately identify, speciate and analyze the antibiogram and formulate hospital infection control guidelines to tackle the menace of these emerging organisms.

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