



NON DIPHTHERIAL CORYNEBACTERIUM SPECIES ISOLATION AND THEIR SUSCEPTIBILITY PROFILE FROM VARIOUS CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL OF EASTERN INDIA.

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ABSTRACT

Non-diphtherial *Corynebacteria* also known as Diphtheroids is being increasingly reported with invasive nosocomial diseases. Little is known about *Corynebacteria* infection in hospital environment of developing country like India. This prospective study was performed to determine the frequency of different species from clinical sources and the susceptibility profiles of different *Corynebacteria* isolated from patients of a tertiary care hospital of eastern India. A total of 150 isolates of different *Coryneform* species were studied here. The isolates were identified to species level by conventional methods and susceptibility profiles were determined. *Corynebacterium amycolatum* was the predominant species (26.67%) in our series followed by *C.pseudodiphtheriticum* (14%). More than 35% of the isolates were resistant to oxacillin, clindamycin, penicillin, gentamicin, and ciprofloxacin while all were exclusively sensitive towards vancomycin, linezolid, and tigecycline. Our study showed that Non-diphtherial *Corynebacterium* species are important cause of nosocomial multidrug resistant infections and continuous efforts to accurate identification at the species level and regular surveillance of their resistant patterns are necessary.

Keywords: Non-diphtherial *Corynebacteria*, Nosocomial, Multidrug resistant infections.

INTRODUCTION

Non-diphtherial *Corynebacteria* also known as Diphtheroids is being increasingly reported as a nosocomial pathogens. Diphtheroids often have been associated with invasive diseases like catheter associated blood stream infection, endocarditis, prosthetic valve endocarditis, meningitis, neurological shunt infection, brain abscess, peritonitis, osteomyelitis and respiratory tract infection particularly in immune suppressed individuals [1].

Species like *C.amycolatum*, *C.jejkeium*, *C.minutissimum*, *C.urealyticum* and *C.striatum* are being reported with increasing frequency in recent years [2,3,4]. Most often these organisms are treated as mere contaminants in the laboratory setting without proper clinical and microbiological analysis [5]. Until recently

the Clinical Laboratory Standard Institute (CLSI) had not published any specific guidelines for the susceptibility testing of *Coryneform* bacteria except recently the CLSI has proposed a reference microdilution method for testing these organisms. Because of emerging antimicrobial resistance in various species there is also an additional need for proper identification of *Corynebacteria* organisms at the species level [6].

Little is known about *Corynebacteria* infection in hospital environment, especially in developing country like India. Thereby this prospective study was performed to determine the frequency of different species from clinical sources and the susceptibility profiles of different *Corynebacteria* isolated from patients of a tertiary care hospital of eastern India.

MATERIAL & METHODS

This prospective study was carried out during the period of December 2012 to April 2014. The *Corynebacteria* organisms studied were isolated from patients admitted in different departments. Collected specimen was inoculated on 5% sheep blood agar, MacConkey's agar with additional chocolate agar for cerebrospinal fluid (CSF) and respiratory samples. *Coryneform* organisms were considered significant and processed further if they were grown in any quantity from normally sterile body fluid or when they were isolated in predominant and pure culture form. In case of urine a repeat test was performed when the first sample grew the organisms as a pure growth with colony count 10^4 /ml or as a predominant growth with total count of 10^5 CFU/ml. A further selection was based on relevant information's obtained from physician attending the patients like fever, presence of indwelling devices, exposure to antibiotics and underlying co morbid conditions. From the collected samples except blood a direct gram stained smear was done. *Coryneform* bacteria grown from the samples were gram stained to see the characteristic morphology. The scheme used for speciation of Diphtheroids was based on biochemical tests as described by different workers (Brazil) which included gram stain morphology, pigmentation and hemolysis around the colonies, a catalase test, test for fermentation or oxidation, nitrate reduction, urea hydrolysis, esculin hydrolysis, lipid requirement, DNA and pyrazinamidase production, gelatin hydrolysis, CAMP reaction and acid production from glucose, maltose, sucrose, xylose, mannose, fructose, mannitol, trehalose, galactose and arabinose.

Antimicrobial susceptibility was determined by disc diffusion method on 5% sheep blood agar in case of non-lipophilic diphtheroids and with 1% added Tween 80 for testing susceptibility for lipophilic coryneform species. Antibiotics discs used for susceptibility testing were: ampicillin (10ug), amoxicillin+clavulanic acid (20/10ug), cefoperazone+sulbactam (75/10ug), ceftriaxone (30ug), chloramphenicol (30ug), clindamycin (2ug), erythromycin (15ug), ciprofloxacin (5ug), gentamicin (10ug), imipenem (10ug), linezolid (30ug), oxacillin (1ug), penicillin (10ug), piperacillin+tazobactam (100/10ug), tetracycline (30ug), ciprofloxacin (5ug), vancomycin(30ug), and tigecycline (15ug). Because of the lack of accepted guidelines by CLSI for disc diffusion method for this group of organisms. The British Society

for Antimicrobial Chemotherapy (BSAC) protocols were followed while testing ciprofloxacin, penicillin and vancomycin [6]. For other antibiotics, CLSI protocols for testing *Staphylococcus aureus* with *S.aureus* ATCC 25923 as control strain were adopted [7,8].

RESULTS

Over a 10-month period investigation a total of 150 isolates belongs to genus *Corynebacterium* were recovered and subjected to speciation. The most frequent sources of these isolates were genitourinary tract (42%), wounds and abscesses (36 %), indwelling catheters (18.66 %), and blood (12%) and respiratory tract specimens (3.33%).(Table 1).

All the isolates obtained from various clinical materials except urine, were in pure form. The most common species isolated from clinical samples were *C.amycolatum* (26.67%), *C.pseudodiphtheriticum* (14%), CDC group G (10.66%), *C.glucorolyticum* (9.33%), *C.minutissimum* (8%), followed by *C.xerosis* (6.6%) and *C.urealyticum* (6%). (Table 2)

Three isolates of *C.pseudodiphtheriticum*, two isolates of *C.minutissimum* and three *C.propinquum* strains were isolated in persons with AIDS. *C.amycolatum* was most frequently isolated species from the urine, indwelling catheter and wound swab. *C.pseudodiphtheriticum* strains were recovered predominantly from genitourinary tract and intravenous site followed by lower respiratory tract and body fluids. The majority of the CDC group G species were recovered from pus samples and catheter tips. *C.xerosis* was predominantly recovered from material obtained from catheter sites, intravenous site and skin wounds. The species of *C.accolens*, *C.striatum*, *C. argentoratense*, *C.propinquum* and *C.pseudodiphthericum* were isolated mainly from samples of blood. Majority of the *C.minutissimum* and *C.urealyticum* strains were isolated from urinary samples.

Table 3 represents the antibiotic susceptibility results presented by *Coryneform* bacterial strains. Most strains were resistant to penicillin, oxacillin, ampicillin, chloramphenicol, erythromycin and more than 30% strains were resistant to ciprofloxacin and gentamicin. Very good activities were shown by vancomycin, linezolid and tigecycline, to which all the isolates were found to be sensitive. *C.jejikeium* and *C.xerosis* isolates were found to be most resistant organisms.

Table 1. Sample wise distribution of isolates (n=150)

Sample	No. of isolates %
Urine	60 (40%)
Pus	36 (24%)
Catheter tips	28 (18.66%)
Blood	18 (12%)
Sputum	5 (3.33%)
Prosthetic secretion	3 (2%)

Table 2. Distribution of the bacterial isolates according to species (n= 150)

Species	No of isolates	% of isolates
<i>C.amycolatum</i>	40	26.67
<i>C. pseudodiphthericum</i>	21	14
<i>C.D.C group G</i>	16	10.66
<i>C.glucoronolyticum</i>	14	9.33
<i>C.minutissimum</i>	12	8
<i>C.xerosis</i>	10	6.6
<i>C.urealyticum</i>	9	6
<i>C.accolens</i>	7	4.66
<i>C.striatum</i>	5	3.33
<i>C.propinquum</i>	4	2.66
<i>C.jejkeium</i>	3	2
<i>C.argentorateneae</i>	2	1.33
<i>C.pseudotuberculosis</i>	2	1.33
Others*	5	3.33

*Others= *C.matruchotti*, *C.afermentans*, *C.imitans*, *C.durans*, *C.afermentans subsp afermentans*

Table 3. Susceptibility profile of isolated Corneyform species

Antimicrobial agents	No. of resistant isolates	Percentage
Piperacillin+tazobactam	34	22.66
Amoxycillin+clavulanic acid	25	14
Cefoperazone+sulbactam	42	28
Chloramphenicol	23	15.33
Ceftriaxone	29	19.33
Clindamycin	67	44.66
Erythromycin	69	46
Ciprofloxacin	56	37.33
Gentamicin	59	39.33
Imipenem	16	10.66
Linezolid	0	0
Oxacillin	79	52.66
Ampicillin	34	22.66
Penicillin	81	54
Tetracycline	23	15.33
Vancomycin	0	0
Tigecycline	0	0

DISCUSSION AND CONCLUSION

Coryneform bacteria are being reported with increasing frequency as potential nosocomial pathogens. With the duration and intensity of immunosuppression that patients are now subject to and increase use of indwelling devices the role of *Coryneform* bacteria became more significant [8,9]. There have been several differences observed among *Coryneform* bacterial species commonly recovered from different clinical materials by several researchers [10]. According to our study the most commonly isolated species was *C.amycolatum*. It was predominantly isolated from genitourinary and blood specimen which is in accordance with previous studies [11,12]. *C.pseudodiphtheriticum* strains were predominantly recovered from i.v. site and genitourinary samples along with few pus samples [13]. The third most common species, accounting for 10.66% of isolates, was

CDC group G isolated in increased frequency from pus and catheter tips. This finding is similar to that of finding of Olender and Ketowska [14].

Corynebacterium minutissimum and *Corneybacterium glucorolyticum* were found in a frequency of 8% and 9.33% each. Both these species were predominantly isolated from urine. All of the *C.urealyticum* species were exclusively found from urine only. *C.propinquum* had been previously recovered from respiratory samples and blood samples. It is in accordance with the findings of similar study conducted elsewhere [15]. *C.tuberculostearicum* was mostly isolated from pus samples. The other species like *C.atypicum*, *C.aurimucosum*, *C.jejkeium*, *C.matruchotii* were isolated less frequently. During last 20 years a renewed interest in *Corynebacterium* species has come out among physicians

and medical microbiologist. Infections due to these groups of organisms are increasing day by day, new species are been described [16-18]. Few recent studies show an increasing rate of antimicrobial resistant to beta-lactam groups, clindamycin, azithromycin, ciprofloxacin, and gentamicin is frequent with vancomycin, linezolid, doxycycline and tigecycline are the most active in vitro [15]. As resistance to many antibiotics is increasing proper antimicrobial susceptibility tests should be done prior institution of therapy.

In our study most strains were resistant to penicillin, oxacillin, erythromycin, ciprofloxacin, ceftazidime, chloramphenicol. Vancomycin, linezolid, and tigecycline showed higher in vitro activity against the different *Coryneform* species. All the isolated species here uniformly sensitive to three above mentioned antimicrobials and many of the isolates were susceptible to imipenem, tetracycline, and ceftriaxone.

C.amycolatum, the predominant isolates showed higher degree of resistance to penicillin, ampicillin, oxacillin, erythromycin, ciprofloxacin and gentamycin. These results are in accordance with previous studies [19,20]. Most of the *C.pseudodiphtheriticum* strains were resistant to penicillin and ampicillin which corresponds to previous studies. In our study all the isolated *Coryneform* strains were susceptible to glycopeptides, tigecycline and vancomycin. Thereby they can be used as first line therapy to treat these serious infections.

Isolates from catheter tips, pus and blood samples showed higher degree of resistance in comparison to isolates from genitourinary tract, sputum and CSF. Multidrug resistant *Coryneform* species were more commonly isolated from indwelling catheter samples [21,22]. The isolates from catheters are potential biofilm former which have got a potential to cause blood stream infection. Most of the CDC group G species in our study were resistant to β -lactam group of drugs. This

finding is in accordance with Weiss et al [23]. Along with β -lactam group of antibiotics it showed higher degree of resistance towards clindamycin, azithromycin and ciprofloxacin. Relatively less resistance was observed to ceftriaxone and imipenem. *C.xerosis* and *C.jejikeium* were the most resistant species within *Coryneform* group tested. *C.glucoronolyticum* strains in the present study were sensitive to the β -lactam group of drugs. Among the isolated species *C.amycolatum*, *C.xerosis*, and CDC group G were the species showed a higher level of resistance to different antimicrobial substances. Isolates from catheter tips showed a higher degree resistance towards antimicrobial agents and majority were multidrug resistant may be because of localized biofilm formation.

Coryneform group of bacteria are nowadays emerging as multidrug resistant nosocomial pathogens in hospital environment. As they are normally present as commensal body flora at one or other body sites caution should be adopted to consider them as a potential pathogens. Proper and series of biochemical tests should be applied to accurately identify them. Molecular diagnostic methodology and sequencing of the isolated *Coryneform* bacteria could be more reliable methodology but because of fund constraints and lack of facility was not feasible in our setup. *Coryneform* group of bacteria are to be considered as a significant pathogens when whenever they are isolated in pure culture or from a sterile body part or isolated repeatedly [3,9]. The aim of our study was to know the frequency of isolation of various species of *Coryneform* group of bacteria and their susceptibility pattern, as almost no information regarding the same was available from this part of India.

Our study showed that several *Corynebacterium* species expresses unpredictable antimicrobial resistant to various antimicrobial substances. Thereby continuous efforts to accurate identification at the species level and regular surveillance of their resistant patterns are necessary.

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