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Isolation of Non-Diptherial Coryne-bacterium (Diphteroids) and its Antibiogram from Various Clinical Samples.

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ABSTRACT

Background: Catalase-positive Aerobic Gram-positive non spore forming bacilli, morphologically resembling Corynebacterium diphthriae, commonly called "diphtheroids" or "coryneform" bacteria are placed under order Actinomycetales. Species of Cornybacterium other than diphtheriae were usually either considered as commensals or saprophytes that are present in human, animal and environment. Among more than eighty species of Corynebacerium identified so far, around 53 species have been associated with human or animal infections. The aim of study is to study the species isolation and antibiogram pattern of diphtheroids from various clinical specimens submitted to the department of microbiology, MRH. **Methods:** All specimens submitted for culture and sensitivity were observed by gram stain and inoculated on Blood agar, Mac conkey agar and Chocolate agar and incubated. Species of Corynebacteriae were identified by biochemical tests like nitrate, urease basic media, and catalase and pyrazinamide reactions. The antibiogram was determined by the disc diffusion method on 5% sheep blood agar. **Results:** Over a period of one year, a total of 2081 various clinical specimens were screened, among which 1206 were culture positive. 445(36.89%) were positive for diphtheroids and 761(63%) were positive for other organisms. Non diphtherial corynebacterial species like, C. amycolatum (40.6%), *C.* pseudo-diphtheriticum (12.1%), C. ulcerans (88%), C. urealyticum (64%), C. propinquum (36%), C. xerosis (4.9%) were isolated and identified. Most of the species were sensitive to antibiotics like Vancomycin(88.7%), Teicoplanin(91%), Linezolid(93%) and Clindamycin (82%). High resistant pattern was shown to antibiotics like Penicillin (83.1%), Amoxyclav (86.9%), Chloramphenicol (69.8%), Gentamicin (68.08%) and Tetracycline (64.04%). **Conclusion:** The present study is an attempt to identify non diphtherial Corynebacterium upto species level and report their antibiogram pattern. Determination of antibiogram of such pathogens is a must, as these species are highly resistant to many of the commonly used antibiotics including Beta-lactams, macrolides, and fluroquinolones. Emergence of multidrug resistance in various species has created the necessity for exact identification of non diphtherial coryneform organisms upto the species level, which helps the clinician to prescribe the antibiotic treatment based on the results of culture and antibiotic sensitivity report.

Keywords: Non diphtherial Corynebacteria (NDC), Isolation, Speciation, Antibiogram.

INTRODUCTION

Non diphtherial Corynebacteria (NDC), which are also referred to as Diphteroids, are a group of aerobic, non-spore bearing gram positive bacilli that are morphologically similar to C. diphtheriae and are placed under the order Actinomycetales which also includes other genera of medical importance, like Mycobacterium, Norcardia and Rhodococcus.[1] Up to now, the pathogenic potential of coryneform bacteria has been underestimated. NDC which are being reported with increasing frequency in recent years, have attracted the attention of clinical microbiologists. These organisms have been associated with invasive disease, particularly in debilitated/immune-compromised patients.

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There are reports of human infections that include urinary tract infections (UTI), infections associated with prosthetic devices, osteomyelitis, septic arthritis, peritonitis, brain abscess, bacteremia and meningitis.[1-4]

Centers for Disease Control and Prevention has published identification schemes and methods for Corynebacterium species and coryneforms, based on phenotypic features of strains from their culture collection and gave Corynebacterium-like taxa provisional "group names".[5-11]

Identification of coryneform bacteria to the species level is often problematic. During the last few years, there has been an increased number of cases reporting an association of coryneform bacteria with disease. Coyle and Lipsky[12] stated that the "recognition of infections caused by coryneform bacteria is highly dependent on the laboratories' ability to identify these species".[12]

For reasons like: (i) the laboratories cannot rely entirely on the databases of commercial identification systems, because they may be incorrect or cover a limited number of taxa only; (ii) the methods used for

identification were inappropriate (e.g., because of lack of chemotaxonomic investigations); (iii) there have been significant changes in the taxonomy of coryneform bacteria; and (iv) the distinction between colonization and infection has not been made in every case. Therefore, there is a need to critically review recent case reports claiming an association of certain coryneform bacteria with disease, and to have the minimal microbiological requirements for publications on associations of coryneform bacteria with disease.[5] Species like Corybacterium amycolatum, C. xerosis, C. pseudodiphtheriticum, C. ulcerance, C. propinquum, C. urealyticum are being reported in increasing frequencies in recent times. These species of corynbacteria are generally considered as saprophytes or laboratory contaminants of clinical materials or specimens, hence it is difficult to correctly decide if recovery of such bacteria implies contamination or has clinical relevance.[13]

Therefore, it is recommended that these organisms should be identified to the genus and species level whenever they grow in pure culture from clinical specimens and/or when they represent the predominant organisms in normally sterile samples.[5] Since standard guidelines for antimicrobial susceptibility testing of Corynebacteria were not available, previously, these tests were carried out by following either CLSI criteria for Staphylococcus or Streptococci or the British Society for Antimicrobial Chemotherapy (BSAC).[15] Disk diffusion method, broth micro-dilution, agar dilution method and epsilometer (E-test) have been tried by many authors.[8,14] Recently, CLSI has proposed reference guideline for antimicrobial susceptibility testing for Corynebacterial species.[16,17]

Non diphtherial corynebacterium appear to be significant pathogens of tropical countries rather than of temperate areas.[18] Vast studies explaining the detailed description which includes the organism's speciation in Indian laboratory setup was absent, hence we had to depend on the data available from the work done in Western countries.

Very little information and few studies till now have been carried out related to the isolation and identification of non diphtherial corynebacterium in the developing countries like India. With the available information, it is difficult to prove the non diphtherial corynebacterium species as a pathogen.

The present study is an effort to isolate, identify and to report the antibiogram of the non diphtherial corynebacteria which are isolated from the clinical materials.

MATERIALS AND METHODS

A prospective study conducted on various samples submitted to Department of Microbiology, Mallareddy Hospital for a period of one year. The organisms were recovered from clinical specimens submitted for culture from patients attending outpatient department and those who were hospitalized. Specimens like urine, pus, sputum, blood, vaginal and prostatic secretions & throat swabs (upper respiratory tract infection-URTI), eye swabs (conjunctivitis), ear swabs (chronic suppurative otitis media- CSOM), cervical swabs(cervicitis), surgical wound swabs (surgical site infection), joint fluid (synovitis), ascitic fluid, pleural fluid, drains and catheter samples from different sites were initially examined by direct microscopy after gram's staining and then inoculated on Blood agar, Mac conkey's agar and chocolate agar. Plates were incubated for 18-24 hours at 37°C.

The significance of the isolates as a pathogen was proved by collecting repeat samples in case of urine specimen if NDC was isolated from the first sample as pure growth, at a count of 10^4 CFU/ml, or as a predominant organism with a count of 10^5 CFU/ml.[5,6] In case of other specimens presence of gram positive rods in direct smear from the sample and growth of similar NDCs in the culture incubated overnight were considered as significant[6]. A repeat sample was collected from same patient on the same day after observing gram stained smear showing Gram positive rods. The plates were incubated for 24-48 hrs. Tiny pinpoint colonies were seen on Blood and Mac conkey agar suggesting gram of Corynebacteria different species of non corynebacteriae were identified by biochemical tests like nitrate, catalase, urease, pyrazinamide tests as explained by Von Graevenitz and Funke.[5]

Antibiotic sensitivity test was performed by on 5% sheep blood agar by using kirby bauer disk diffusion method by standardizing the inoculum at 0.5 Mc Farlands. Antibiotics used were: Ampicillin (10 µg), amoxycillin-clavulanic acid (20/10 µg), ceftriaxone (30 µg), chloramphenicol (30 µg), clindamycin (2 µg), erythromycin (15 µg), ciprofloxacin (5 µg), nitrofurantoin (300 µg), Tobramycin (10 mcg), gentamicin (10 µg), imipenem (10 µg),linezolid (30 µg), oxacillin (1 µg), penicillin (10 units), piperacillin-tazobactum (100/10 µg), tetracycline (30 µg), vancomycin (30 µg) and tigecycline (15 µg).[8] The Quality control strains used were Staphylococcus aureus ATCC 25923. Due to lack of established Clinical and Laboratory Standards Institute (CLSI)[9] guidelines for this group of organisms, the results were interpreted based on a combination of CLSI guidelines applicable for *S. aureus* and the British Society for

Antimicrobial Chemotherapy guidelines for testing ciprofloxacin, penicillin and vancomycin.

RESULTS

In one year study, out of total 2081, including all types of clinical specimens submitted for culture and sensitivity in microbiology laboratory, 875 (42.04%) were culture negative and 1206 (57.9%) samples were culture positive showing growth of one or more organism. Among which 761 (63.1%) samples were showing mixed growth of non-diphtheritic corynebacterial species and other organisms like Staphylococcus species, Coagulase negative Staphylococcus, Escherichia coli, Klebsiella species, Proteus spp. other Enterobacteriaceae species & Pseudomonas species. Pure growth of NDC was seen in 445(36.8%). The most common species of Corynebacterium isolated from the clinical sample was C. amycolatum 181(40.6%), followed by C. ulcerans 88 (19.7%), C. urealyticum 64 (14.3%), C. pseudodiphtheriticum 54 (12.1%), C. propinquum 36 (8.08%), and C. xerosis 22 (4.9%). The number of NDCs isolated from various specimens 195 (43.8%) from Urinary tract infections, 147 (33.0%) from wound swabs/ surgical sites, 37(8.31%) from blood, 27 (6.06%) from catheter specimen, 15 (3.3%) from Respiratory tract, 12 (2.6%) from Genital(prostatic/vaginal secretions), 5 (1.1%) from eye/ear, 5 (1.1%) from Pleural fluid, 2 (0.4%) from ascetic fluid, and no isolate from Synovial fluid.

Variable antimicrobial sensitive and resistant pattern were seen. There was an overall high sensitivity of the isolates to Vancomycin (88.7%), Teicoplanin (91%), Linezolid (93%), and Clindamycin (82%). Resistance was exhibited by isolates towards Penicillin (83%), Amoxyclav (86.9%), Chloramphenicol (69.8%), Gentamicin (68.08%), Tetracyclin (64.04%). More than 50% of isolates were resistant to Ampicillin & Nitrofurantoin, and more than 40% isolates were resistant to Ofloxacin, Oxacillin, and Erythromycin [Table 1-4].

Table 1: Non diphtherial corynebacteria species frequently isolated from different clinical materials.

Organism	Clinical Material
C. amycolatum	Blood, Wound, peritonitis, cellulitis, sepsis, endocarditis
C. pseudodiphtheriticum	Respiratory specimens after Pneumonia, Exudative pharyngitis, blood, wound, keratitis, conjunctivitis, urine, peritoneal fluid, cervical necrosis, ear, synovial fluid
C. proppinquum	Blood cultures, respiratory specimens, endocarditis, osteon synthesis aspirate, pleural effusion
C. ulcerans	Pharyngitis, sinusitis, tonsillitis, pulmonary nodule, skin ulcers.
C. urealyticum	UTI, blood cultures, endocarditis, respiratory specimens associated with pneumonia, soft tissue infections
C. xerosis	Ear, brain abscess, osteomyelitis, arthritis

Table.2:. Preliminary biochemical tests of non diphtherial corynebacterium species isolated

Organisms	Biochemical tests			
	Nitrate test	Urease test	Catalase Test	Pyrazinamide test
C[d]. amycolatum	V[a]	V	+[b]	+
C. xerosis	V	-[c]	+	+
C. pseudodiphtheriticum	+	+	+	+
C. ulcerans	-	+	+	-
C. propinquum	+	-	+	V
C. urealyticum	-	+	+	+

a – variable, b –positive, c –negative, d – Corynebacterium

Table 3: Percentage of NDCs Isolated from Different Clinical Specimens

Clinical specimens	No. of Isolates (%)
UTI	195(43.8%)
Wound swabs/ surgical sites	147(33%)
Blood	37(8.31%)
Catheter specimens	27(6.06%)
Respiratory tract infections	15(3.3%)
Genital (prostatic/ vaginal secretions)	12(2.6%)
Eye/ear	5(1.1%)
Pleural fluid	5(1.1%)
Ascitic fluid	2(0.4%)
Synovial fluid	Nil
Total	445

Table 4: No. of NDCs isolated from different clinical specimens

Organism	No. of Isolates (n=445)	% of Isolates
C. amycolatum	181	40.6%
C. ulcerans	88	19.7%
C. urealyticum	64	14.3%
C. pseudodiphtheriticum	54	12.1%
C. propinquum	36	8.08%
C. xerosis	22	4.9%
Total	445	100%

Table 5: Antimicrobial Sensitivity and Resistant Pattern of the Isolates

Antibiotic	Sensitive	% of sensitivity	Resistant	% of Resistant
Ampicillin	207	46.5%	238	53.4%
Amoxyclav	58	13%	387	86.9%
Chloramphenicol	134	30.1%	311	69.8%
Ciprofloxacin	289	64.9%	156	35.05%
Ceftriaxone	289	64.9%	156	35.05%
Clindamycin	365	82%	80	17.9%
Erythromycin	267	60%	178	40%
Gentamicin	142	31.9%	303	68.08%
Linezolid	414	93%	31	6.9%
Nitrofurantoin	213	47.8%	232	52%
Ofloxacin	245	55%	200	44.9%
Oxacillin	262	58.8%	183	41.7%
Penicillin	75	16.8%	370	83.1%
Teicoplanin	405	91%	40	8.9%
Tetracycline	160	35.9%	285	64.4%
Vancomycin	395	88.7%	50	11.2%

DISCUSSION

The total culture positive percentage in our study among 2081 clinical specimens, was 1206 (57.9%). Pure growth of NDC among 1206, culture positive cases was 445 (36.8%). NDC s among all the clinical specimens were frequently isolated from urinary tract infections (43.8%) followed by wound and surgical site infections (33%), blood (8.31%), catheter specimen (6.06%), Respiratory tract infections (3.3%) , Genital (prostatic/vaginal secretions) (2.6%), eye/ear (1.1%), Pleural fluid 5 (1.1%) & ascitic fluid 2(0.4%). These percentage of isolation was nearly in correlation with percentage shown by Reddy BS[8] (37.3%) in case of Urinary specimens and Mathavi et al[18] (45.24%) in case of surgical site infections. The commonly isolated non diphtherial corynebacterium in our study were C. amycolatum (40.6%), followed by C. ulcerans (19.7%), C. urealyticum (14.3%), C. propinqum (8.08%), C. xerosis (4.9%).

C. amycolatum was also the most commonly isolated NDC by Lagarou et al[20](53%) followed by C. jeikeium (12%), C. straitum (8%), C. afermentans (7%), C. minutissium (6%), C. urealyticum (3%), C. glucoronolyticum (0.7%) and C. xerosis (0.7%). Whereas Olender A et al[21]showed C. amycolatum as (29.2%) followed by C. striatium (16.7%), C grp G (16.7%), C. jeikeium.

Studies by T.C.F. Camello et al[19], also reported C. amycolatum (29.55%) and *C.* pseudodiphtheriticum (13.65%) as the most predominant organisms isolated. Our percentage and pattern of isolation of different species of non diphtherial corynebacteria is different when compared with work done by other authors. This might be due to differences in the geographical distribution of the organism and also due to lack of definite identification and isolation schemes in the laboratories where these studies were carried out.

Variable antimicrobial resistance/susceptibility patters have been observed from the studies conducted previously.[22]

The overall resistance pattern of the isolates in the present study is depicted in the Table. 5. It shows high resistance percentage against Penicillin (83.1%), Amoxyclav (86.9%), Tetracycline (64.04%), Gentamicin (68.8%), Chloramphenicol (69.8%), Ampicillin (53.4%). Whereas the isolates show high sensitivity against Clindamycin (82%), Vancomycin (88.7%), Teicoplanin (91%), Linezolid (93%), Ofloxacin (55%). The resistant pattern of the antibiotic in our study was in near accordance with results presented by Reddy et al[8], Mathavi S et al[19] and Lagarou et al.[20]

Out sensitivity pattern results correlated well with the sensitivity results shown by Meher Rizvi et al[23], in which the percentage of their isolates which were sensitive to vancomycin (92.1%), Teicoplanin (92%),

Linezolid (100%). Reddy et al[8], also showed 100% sensitivity to Vancomycin and Linezolid.

CONCLUSION

The isolation and identification of Non diphtherial Coryneform bacteria remains a challenge for regular diagnostic laboratories, this may be due to many number of species which are included in this group, in addition to it, due to lack of well-established identification schemes. In recent times these group of organisms are not considered as commensals, this is because of their repeated isolation, especially from clinical samples from immune-compromised and debilitated cases/ patients. Non diphtherial Corynebacteria are to be considered as clinically significant organisms in case where they are isolated in pure cultures, or isolated from a sterile site, or isolated repeatedly.[6] Species identification and isolation of organism like C. amycolatum and other species, which are most commonly and increasingly recovered from clinical specimens and considered as direct pathogens, especially in immune-compromised and hospitalized patients, is necessary. Determination of antibiogram of such pathogens is a must, as these species are highly resistant to many of the commonly used antibiotics including Beta-lactams, macrolides, and fluroquinolones. Emergence of multidrug resistance in various species has created necessity for exact identification of non diphtherial coryneform organisms to the species level. The present study is an attempt to isolate these organism to species level and predict their antibiogram, which helps to improve and modulate the empirical treatment after the results of culture and antibiotic sensitivity testing.

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