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Non Diphtheritic Corynebacteria (NDC) and Their Clinical Significance: Clinical Microbiologist's Perspective

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Abstract Aerobic, Gram positive, catalase positive and non-spore forming bacilli, which are morphologically similar to *Corynebacterium diphtheriae* are described as either diphtheroids or coryneform bacteria, resembling *C diphtheriae*. Corynebacteria are a group of bacteria placed under the family *corynebacteriaceae,* which come under the phylum, Actinobacteria. Among the members of genus *Corynebacterium,* only *C diphtheriae* is considered as a pathogen but other species are present either as normal flora in human or as saprophytes in the environment and have rarely been associated with human infections. Of late, there have been increased reports of both new species of genus *Corynebacterium* and their occurrence in various human infections. It is now imperative that clinical microbiologists and clinicians understand the potential role of NDC in human infections. Only few studies globally have characterized the human clinical isolates of NDC and their antimicrobial susceptibility patterns. This review tries toexamine? the potential pathogenic nature of NDC, which warrants their identification and prompt reporting when isolated from human clinical specimens.

Keywords: *clinical microbiology, Non-diptheritic Corynebacteria, Medically important Corynebacterial species*

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1. Introduction

Corynebacterial species other than *Corynebacterium diphtheriae* are a group of aerobic, non-spore bearing Gram positive bacilli that are morphologically similar to *C diptheriae*. The genus *Corynebacterium* is placed under the order Actinomycetales which also includes other genera of medical importance, like *Mycobacterium, Nocardia* and *Rhodococcus* [1]. Non diptheritic corynebacteria (NDC) are usually either commensals or saprophytes that are present in human, animal and environment. Few NDC including *C glutamicum*, and *C feeiciens* are recognised for their biotechnological significance in the production of essential amino acids and vitamins [2]. Among more than eighty species of Corynebacterium identified so far, around 53 species have been associated with human or animal infections [3]. NDC are occasionally isolated from human clinical samples but have most often been ignored as laboratory contaminants. Isolated reports of human infections with non diptheritic corynebacteria mostly in debilitated/immunocompromised patients have attracted the attention of clinical microbiologists [4,5]. Although the pathogenicity of NDC is still in debate, there are several reports of human infections that include urinary tract infections (UTI's), infections associated with prosthetic devices, osteomyelitis, septic arthritis, peritonitis, brain abscess, bacteremia and meningitis [1,4,5,6]. There have been several instances where NDC have been isolated from skin and wound infections, either singly or in the presence of other bacterial species, but in such cases, their virulence has been ignored as they have been seen as either commensals or laboratory contaminants by many clinical microbiologists. Recent increase in the reports of human infections with NDC has prompted clinical microbiologists to consider them as potential pathogens when isolated from sterile sites and when a duplicate sample from the same site confirms the growth.

2. Medically Important Corynebacterial Species

Medically important Corynebacterial species are classified as non-lipophilic and lipophilic corynebacteria. C amycolatum, C argentoratense, C pseudodiphtheriticum,

C ulcerans, C minutissimum, C striatum, C xerosis, C glucuronolyticum, C coylae, C frenyi, C atypicum, C matruchotii, C falsenii, C confusum, C afermentans and C propinquum constitute non-lipophilic corynebcteria.[3] Lipophilic corynebacterial species include C jeikium, C accolens, C afermentans sub spp. lipophillum, C urealyticum, C tuberculosteraicum and CDC group G [9,10,11,12]. Recently, clinical laboratories throughout the world have been reporting NDC from various human clinical samples. C pseudotuberculosis, C riegelii, C singular, C sundsvallense, C thomessenii, C imitans, Ckroppenstedtii, C lipophiloflavum, C mycetoides, C epidermicanis, Cmucifaciens, C accolens, C macginleyi, C durum, C ureicelerivorans, C simulans, C pilbariense and C freiburgense are few of the other NDC that are medically significant [13,14,15,16]. Other bacteria that belong to the genus Corynebacterium, isolated from animals (cattle, sheep, pig, mouse, vole, dog, seal, horse and monkey) but are not associated with human infections include C auriscanis, C bovis, C camporealense, C cystitidis, C kutscheri, C mastitidis, C phocae, C pilosum, C acetoacidophilum, C cervicis, C crenatum, C fastidiosum, C genitalium, C nephridii, C nigrans (black pigment producing), C pseudogenitalum, C segmentosum and C thermoaminogenes [17]. Although only few studies have been done on the isolation and characterization of NDC from human specimens, it has been observed that there is variation in the species isolated from different geographical regions. Among the studies that characterized NDC from human specimens, C amycolatum, C jeikium, C striatum, C minutissimum, C urealyticum, C pseudodiptheriticum and CDC group G have been most frequently isolated [18,19].

3. Laboratory Identification

Laboratoty Identification of NDC from human clinical samples can be performed using various methods that include conventional (morphology, Gram's stain, cultural and biochemical characters), chemotaxonomic and molecular techniques. The members of the genus *Corynebacterium* have been identified and amendedover many years with more and more novel species. The core description of the species is done based on 16S r RNA gene sequence analysis and specific chemotaxonomic, morphological and phenotypic characters that included G+C content, cell wall composition (meso-diaminopimelic acid, short-chain mycolic acid, palmitic acid oleic acid, stearic acid, tuberculostearic acid, arabinose and galactose) [20]. Conventional methods for the laboratory identification of NDC in regular practice include culture Figure 1, gram's stain Figure 2, catalase, oxidase, motility, tween 80 hydrolysis, gelatine hydrolysis, casein hydrolysis, tyrosine and xanthine utilization, nitrate reduction test, cAMP test, esculin hydrolysis, urease test, oxidative/fermentative (OF) test, alkaline phosphatase test, pyrazinamidase test, tuberculostearic acid test and carbohydrate fermentation tests [21,22]. Although Corynebacteria have no exacting growth requirements, use of cystine-tellurite blood agar, supplementation of fosfomycin and tween 80 (0.1-1%) to blood agar, improves the growth of clinically significant NDC [27].

Figure 1. Growth on blood Agar showing white to cream colour non-haemolytic colonies

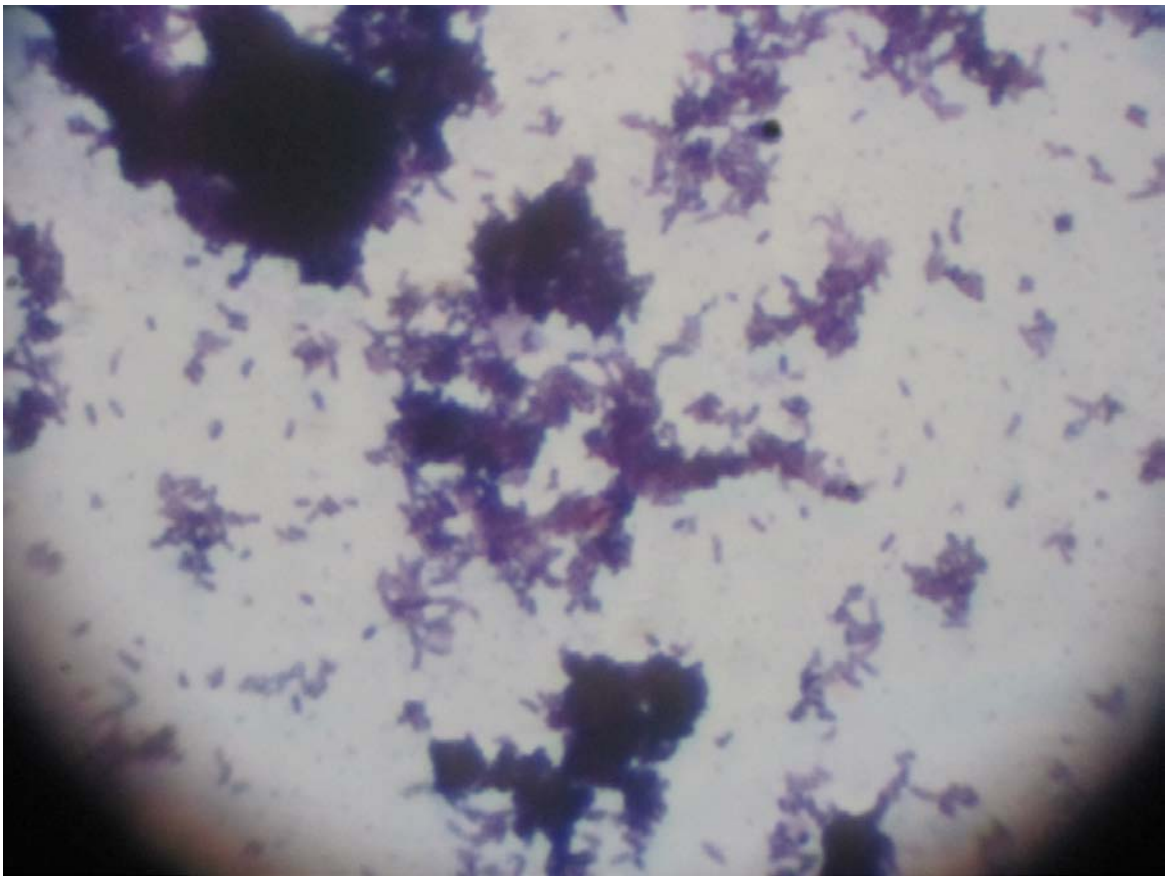

Figure 2. Gram's stain showing gram positive bacilli

Among the automated identification systems available recently API coryne strip, API CH 50 plus, API 20E (API Biomeirieux, France), BBC crystal, Rap ID ANA II, Rap ID CB plus (Remel, Inc., Lenexa, KS, USA), Vitek ANI card, VITEK 2 ANC card (Biomerieux) and biolog system (Biolog, Hayward, CA USA) [24]. Other methods of identification include chemotaxonomic system (analysis of cellular fatty acid (CFA) levels) and polyphasic approach that includes conventional system, CFA and cell wall analysis [25]. Another advanced method for identification of NDC is the use of matrix- associated laser- induced desorption iolnization (MALDI)-time of flight Mass Spectroscopy (MALDI-TOF MS) [26]. Computer softwares like EDGAR (evolutionary divergences in Gegenees) and pathogenic island identification for pathogenecity (PIP)) have been recently reported to be available for characterization of genus *Corynebacterium* [27].

CoryneBase, a genomic database for *Corynebacterium* that provides annotated genome sequence of *Corynebacterium* including 165,918 coding sequences, 4180 RNA's, advanced bioinformatic analysis tools for homology search, virulence factor database (VFDB), pairwise Genome comparision (PGC) for comparative genomic analysis and pathogenomic profiling tool (PathoPro T) for pathogenomic analysis is now available online with facility of web interface at http://corynebacterium.um.edu.my/ [28].

4. Antimicrobial Susceptibility

Previously there was no specific guideline for the characterization and antimicrobial susceptibility testing of NDC. Disk diffusion method, broth microdilution, agar dilution method and epsilometer (E-test) have been tried by many researchers in the past [29,30]. The reason for the absence of specific guideline for sensitivity testing of NDC is that some NDC are fastidious and require exacting growth requirements. Previous studies on antimicrobial susceptibility of NDC have either used Clinical Laboratory Standards Institute)(CLSI) criteria for *Staphylococci* or *Streptococci* or the British Society for Antimicrobial chemotherapy (BSAC) [31].Recently, the CLSI has published guideline for test conditions and interpretative criteria for antimicrobial susceptibility testing of Corynebacterial species [32,33].

Variable antimicrobial resistance/susceptibility patterns have been observed from the available literature [34]. More than 80% of the human isolates were sensitive to aminoglycosides (Amikacin and gentamicin)ref, 60% 0f the isolates were sensitive to oxacillin and ciprofloxacin. Greater percent of resistance was recorded against penicillin, erythromycin and clindamycin. Vancomycin, linazolid, quinapristin and dalphipristin were the antibiotics most effective against NDC. Variable susceptibility patterns were noted against co-trimoxazole, nitrofurantoin, teicoplanin, doxycycline, norfloxacin and cephalosporins [34]. Occurrence of betalactamase producing, multidrug resistant bacteria was also noted in some previous studies [34,35,36].

5. Recent Trends

Recognition of NDC as potential pathogens appears to be logical with increasing reports of both mild and serious infections globally. A recent study has noted that a

member of NDC *C striatum*, a multi-drug resistant species was responsible for an outbreak of nosocomial infection in a Belgian hospital. This isolate was confirmed by 16SrRNA gene sequencing, MALDI-TOF MS, polymerase chain reaction (PCR) and pulse-field gel electrophoresis (PFGE) [37]. Another recent report about NDC causing native joint septic arthritis has highlighted the multi-drug resistant nature of the bacteria. This supposed pathogen was confirmed by mass spectroscopic and nucleic acid based assays [38]. A first case of infective endocarditis in a child, due to *C propinquum* was reported recently, emphasizing its significance among paediatric infections [39]. Another study reported that healthy human skin is colonized with macrolide, lincosamide and streptogramin B resistance (MLS_B) - carrying resistance genes (erm (A), erm (B), erm (C), erm (X), lin (A), msr (A) and mph (C)) [40]. Isolated and confirmed reports recently of bacteremia in a leukaemia patient caused by a multi-drug resistant strain (*C resistens* DSM 45100), bacteremia in an infant on vancomycin therapy caused by C falsenii and pseudomembranous nectrotizing tracheitis secondary to *Corynebacterium* species should be considered as an alarming bell for recognizing NDC's as potential pathogens [41,42,43]. Recently there are increasing reports of novel species of Corynebacteria which in future may be associated with human infections [44].

6. Conclusion

Non diphtheritic corynebacteria (NDC) are a group of coryneform bacteria which are morphologically similar to *C diptheriae*. Many clinical microbiology laboratories consider isolation of these bacteria in human specimens as a sign of contamination. The characterization and antimicrobial susceptibility of human clinical isolates of NDC is not adequately studied throughout the world. Though these bacteria are present normally in human (especially skin and upper respiratory tract), in animals and are also present in the environment as saprophytes, the pathogenicity of NDC and their role in infection remains least understood. Isolation of NDC in clinical specimens from immunocompromised and debilitated/immunocompromised patients, isolation from normally sterile sites of human body and repeated isolation of these bacteria from various clinical samples confirm their role in infection. Increasing reports of new species of NDC both from the environment and from human specimens, association of these bacteria with human, animal and animal to human infections warrant careful attention of clinicians, clinical microbiologists and veterinarians to consider them as potential pathogens.

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References

[1] Dorella FA, Pacheco LGC, Oliveira SC, Miyoshi A and Azevedo V. Coryne bacterium pseudotuberculosis: microbiology, biochemical properties, pathogenesis and molecular studies of virulence. Vet Res. 2006; 37: 201-218

[2] Kalinowsky J Bathe B, Bartels D, Bischoff N, Bott M, Burkovsky A, et al. The complete Corynebacterium glutamicum ATCC 13032 genome sequence and its impact on the production of L-aspartate-derived amino acids and vitamins. J Biotechnol 2003; 104: 5-25.

[3] Bernard KA and G Funke. Genus Corynebacterium,. In: WB Whitman, M Goodfellow, P Kampfer, HBusse, ME Trujillo, W Ludwig, K Suzuki and A Parte (eds.), Bergey's manual of systematic bacteriology. 2012; 5: p. Start1-end Springer, New York.

[4] Casella, P., M. A. Bosoni, and A. Tommasi. 1988. Recurrent Corynebacterium aquaticum peritonitis in a patient undergoing continuous ambulatory peritoneal dialysis. Clin. Microbiol. Newsl. 10: 62-63.

[5] Tendler, C., and E. J. Bottone. 1989. Corynebacterium aquaticum urinary tract infection in a neonate, and concepts regarding the role of the organism as a neonatal pathogen. J. Clin. Microbiol. 27: 343-345.

[6] Beckwith, D. G., J. A. Jahre, and S. Haggerty. 1986. Isolation of Corynebacterium aquaticum from spinal fluid of an infant with meningitis. J. Clin. Microbiol. 23: 375-376.

[7] Oleander A, Ketowska I. Wound infections due to opportunistic corynebacterium species. Med Dosw Mikrobiol 2010; 62: 135-140.

[8] Kaplan, A., and F. Israel. 1988. Corynebacterium aquaticum infection in a patient with chronic granulomatous disease. Am. J. Med. Sci. 296: 57-58.

[9] Funke, G., G. Martinetti Lucchini, G. E. Pfyfer, M. Marchiani, and A. von Graevenitz. 1993. Characteristics of CDC group 1 and group 1-like coryneform bacteria isolated from clinical specimens. J. Clin. Microbiol. 31: 2907-2912.

[10] M. Aravena-Roman,C. Sproer, B. Straubler,T. Inglis and A. F. Yassin. Corynebacterium pilbarense sp. nov., a non lipophilic corynebacterium isolated from a human ankle aspirate International Journal of Systematic and Evolutionary Microbiology 2010; 60: 1484-1487

[11] Colt, H. G., Morris, J. F., Marston, B. J. & Sewell, D. L. (1991). Necrotizing tracheitis caused by Corynebacterium pseudodiphtheriticum : unique case and review. Rev Infect Dis 13, 73-76.

[12] Funke, G., Lawson, P. A. & Collins, M. D. (1997a). Corynebacterium Mucifaciens sp. nov., an unusual species from human clinical material. Int J Syst Bacteriol 47, 952–957

[13] Neubauer, M., Sourek, J., Ryc, M., Bohacek, J., Mara, M. & Mnukova, J. (1991). Corynebacterium accolens sp. nov., a gram-positive rod exhibiting satellitism, from clinical material. Syst Appl Microbiol 14, 46-51.

[14] Riegel, P., Ruimy, R., de Briel, D., Pre vost, G., Jehl, F., Christen, R. & Monteil, H. (1995). Genomic diversity and phylogenetic relationships among lipid-requiring diphtheroids from humans and characterization of Corynebacterium macginleyi sp. nov. Int J Syst Bacteriol 45, 128-133.

[15] Yassin, A. F. (2007). Corynebacterium ureicelerivorans sp. nov., a lipophilic bacterium isolated from blood culture. Int J Syst Evol Microbiol 57, 1200-1203.

[16] Guido Funke, Reinhard Frodl, Kathryn A. Bernard and Ralf Englert. Corynebacterium freiburgense sp. nov., isolated from a wound obtained from a dog bite. Int J Syst Bacteriol 2009 vol. 59 no. 8 2054-2057.

[17] Alexander Von Graevenitz and Kathryn Bernard. The genus Corynbacterium-Medical. Prokaryotes 2006; 3: 819-842.

[18] Kathryn Bernard. The genus Cirynebacterium and other medically-relevant, coryneform like bacteria. J Clin Microbiol.

[19] Coyle, M. B. & Lipsky, B. J. (1990). Coryneform bacteria in infectious diseases: clinical and laboratory aspects. Clin Microbiol Rev 3, 227-246.

[20] Bernard, K. A., M. Bellefeuille, and E. P. Ewan.. Cellular fatty acid composition as an adjunct to the identification of asporogenous, aerobic gram-positive rods. J. Clin. Microbiol. 1991; 29: 83-89.

[21] Funke G Bernard KA. Coryneform Gram-positive rods. In: Murray PR, Baron EJ, Editors 8th Ed. Manual of Clinical microbiology. 2003; 38: 472-496, ASM press Washington.

[22] Fernandez-Natal MI, JA Saez-Nieto, S Valdezate, RH Rodriguez-Pollan, S Lapena, F Cachon and F Soriano. Isolation of Corynebacterium ureiceleriovorans from normally sterile sites in humans. Eur J Clin Microbiol Infect Dis 2009; 28: 677-681.

[23] Funke G and K A Bernard. Coryneform Gram-positive rods. In: Manual of Clinical Microbiology 10th Ed. (eds.) J Versalovic, K C Carroll, G Funke, J H Jorgensen, M L Landry and D W Warnock. ASM press Washington DC 2011; 1: 4123-442.

[24] Rennie RP, Borosnikoff C, L Turnbull, L B Reller, S Mirrett, W Janda, K Ristow and A Kirlcich. Multicenter evaluation of Vitek 2 anaerobe and Corynebacterium identification card J Clin Microbiol 2008; 46: 2646-2651.

[25] Hollis, D. G., and R. E. Weaver. 1981. Gram-positive organisms: a guide to identification. Special Bacteriology Section, Centers for Disease Control, Atlanta.

[26] Gaillot O, N Blondiaux, CLoiez, F Wallet, N Lemaitre, S Herwegh and R J Courcol. Cost-effectiveness of switch to matrix-assisted laser desorption ionization-time of flight mass spectrosmetry for routine bacterial identification. J Clin Microbiol 2011; 49: 4412.

[27] S C Soares, R T J Ramos, W M Silva, L C Olivaira, L G Amorim, R Hirata Jr, Et al Coryne bacterium pathogenic species in next-generation genomic era: the use of EDGAR and PIPS software and the importance of pathogenecity islands identification in pan-genomic analyses of pathogenic species. 2013, 1584-1599. http://www.formatex.info/microbiology4/vol3/1584-1599.pdf.

[28] Heydari H, Siow CC, Tan MF, Jakubovics NS, Wee WY, et al. CoryneBase: Corynebacterium genomic resources and analysis tools at your fingertips. Plos One 2014; 9(1): e86318.

[29] Reddy BS, Chaudhury A, Kalawat U, Jayaprada R, Reddy G, Ramana GV. Isolation, speciation and antibiogram of clinically relevant non-diptherial corynebacteria (Diptheroids). Ind J Med Microbiol 2012; 30: 52-57.

[30] Weiss K, Laverdie're M, Rivest R. Comparision of antimicrobial susceptibilities of Corynebacterium species b y broth microdilution and disk diffusion methods. Antimicrob Agents Chemother 1996; 40: 930-3.

[31] Andrews JM. For the BSAC Working party on susceptibility testing. BSAC standardized disc susceptibility testing method (version 8). J Antimicrobial Chemother 2009; 64: 454-489.

[32] Clinical Laboratory Standards Institute. 2010. M45-2A. Methods for Antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria; approved guideline, Volume 30 Number 18. Clinical Laboratory Standards Institute, Wayne, PA.

[33] Clinical Laboratory Standards Institute. 2012. M100-S22. Performance standards for antimicrobial susceptibility testing; twenty-second international supplement. Volume 32, Number 1. Clinical Laboratory Standards Institute, Wayne, PA.

[34] Williams DY, Selepak ST, Gill VJ. Identification of clinical isolates of nondiptherial Corynebacterium species and their antibiotic susceptibility patterns. Diag Microbiol Infect Dis 1993; 17: 23-28.

[35] Byram A, Eksi F, and Balci I. Resistance problem of coryneform bacteria isolated from intensive care unit samples. Res J Microbiol 2006; 1: 136-141.

[36] Turk S, Punab M, Mandar R. Antimicrobial susceptibility patterns of coryneform bacteria isolated from semen. Open Infect Dis J 2009;3: 31-6.

[37] *Verroken, C. Bauraing, A. Deplano, P. Bogaerts, D. Huang, G. Wauters and Y. Glupczynski. Epidemiological investigation of a nosocomial outbreak of multidrug-resistant Corynebacterium striatum at one Belgian university hospital. Clinical Microbiology and Infection* Volume 20, Issue 1, *pages 44–50, January 2014*

[38] Lars F. Westblade,Farah Shams, Scott Duong, Oosman Tariq, Alan Bulbin, Dava Klirsfeld,et al. Septic Arthritis of a Native Knee Joint Due to Corynebacterium striatum. J. Clin. Microbiol. May 2014 vol. 52 no. 5 1786-1788.

[39] Yu Kawasaki, MD, Kousaku Matsubara, Haruko Ishihara, Hiroyuki Nigami, Aya Iwata, Koji Kawaguchi, et al. Corynebacterium propinquum as the first cause of infective endocarditis in childhood. Journal of Infection and Chemotherapy Volume 20, Issue 5, May 2014, Pages 317-319.

[40] Magdalena Szemraj,[1] Anna Kwaszewska,[1] Renata Pawlak,[2] and Eligia M. Szewczyk. Macrolide, Lincosamide, and Streptogramin B Resistance in Lipophilic Corynebacteria Inhabiting Healthy Human Skin. Microb Drug Resist. 2014 Apr 15 PMID:24735183.

[41] Jasmin Schröder, Irena Maus, Katja Meyer, Stephanie Wördemann, Jochen Blom, Sebastian Jaenicke et al. Complete genome sequence, lifestyle, and multi-drug resistance of the human pathogen *Corynebacterium resistens* DSM 45100 isolated from blood samples of a leukemia patient. *BMC Genomics* 2012, 13:141.

[42] Pui-Ying Iroh Tam, Mark A. Fisher and Nancy S. Miller. Corynebacterium falsenii Bacteremia Occurring in an Infant on Vancomycin Therapy. J. Clin. Microbiol. September 2010 vol. 48 no. 9 3440-3442.

[43] Jorge Guerrero, Pavan Mallur, Erik Folch, Colleen Keyes, Isaac E Stillman, Sidhu P Gangadharan. Necrotizing Tracheitis Secondary to Corynebacterium Species Presenting With Central Airway Obstruction. Respiratory Care January 1, 2014 vol. 59 no. 1 e5-e8.

[44] *Sophie Edouard, Fehmida Bibi, Dhamodharan Ramasamy, Jean-Christophe Lagier, Esam Ibraheem Azhar, Catherine Robert,et al.* Non-contiguous finished genome sequence and description of *Corynebacterium jeddahense* sp. nov. Standards in Genomic Sciences Vol 9, No 3 (2013).