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Gemella Morbillorum Incidence within Typhoid Fever Patients

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Abstract

Bacteria colonize different niches of oral cavity including tongue, gingiva, tonsils and hard and soft plates. 302 swabs collected only 67 (22.18%) were primary confirmed utilizing the morphological and biochemical tests as *Gemella morbillorum* as opportunistic pathogen from oral cavity of children infected with typhoid fever. Genetic identification of 16s rRNA of isolated pure culture exemplified the presence of that gene in 533 bp of electrophoresis.

Keywords: *Gemella morbillorum*, 16s rRNA, bacteria and oral cavity.

Introduction

Healthy individuals include many organisms as normal flora especially oral microbiome (Dewhirst et al., 2010) respiratory, intestinal and genitourinary tract and warm blooded animals (Akiyama et al., 2001; Dewhirst et al., 2010; Van De Wijgert et al., 2014; Takayasu et al., 2017). *Gemella* spp. regarded as commensals and sometimes behave as opportunistic. These microorganisms become pathogens in specific perturbations for the host, like infections during injury or disturbances in immune system and elderly (Brown et al., 2012). According to Lamont, (2018), the microbiome is formed when several bacterial communities interact with each other and with the host environment. When the oral microbiome is out of whack, disease-causing bacteria can flourish and cause severe infections.

Other studies conducted the crucial role of microbiota in human status and pathology at molecular and cellular levels (Lozupone et al., 2007.; Shulzhenko et al., 2011; Petrova et al., 2013). Connections among microorganisms, nourishment and immune response touch health conditions. Just obvious in body protection that provided by commensal bacteria through space and feeds competition (Round & Mazmanian, 2009).

Opportunistic pathogens like *Gemella* can cause both localized and systemic infections, the severity of which can vary. Arthritis (Vasishtha et al., 1996), meningitis, bacteremia and endocarditis (Mitchell & Teddy, 1985). Two species belongs to *Gemella* genus are the most

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predominant *Gemella haemolysans* and *Gemella morbillorum* (Berger, 1992). *Gemella morbillorum* react as catalase and cytochrome oxidase negative, facultative anaerobic, gram-positive cocci, genetic content DNA G+C about 30–34(mol%) beside hemolytic ability (Collins & Falsen, 2015).

Close similarity between *Gemella* and *streptococcus viridans* revealed additional factor in clinical laboratory diagnosis. Both are susceptible to (vancomycin and penicillin) with moderate aminoglycosides resistance (Sawada et al., 2009). Study by Woo et al., (2003) disclosed the identity between *Gemella* and *Granulicatella adiacens* specifically when commercial phenotypic instruments were used. Therefore, molecular technique helps significantly in descriptions of them.

Evolutionary development in methods utilizing 16S rRNA extended our knowledge about oral microbiota (Christensen & Ruoff, 2015). Visit www.homd.org to access the Human mouth Microbiome Database, where you may find a catalog of mouth bacteria along with descriptions of their traits and genetic information. Ostensible dissimilarity in tumor and nontumor mucosa microbiome stated via 16S ribosomal RNA methods of Gemella morbillorum (Hooper et al., 2007; Pushalkar et al., 2012).

Salmonella spp., regardless of the species, is the sole causal agent of typhoid. This hazardous microorganism possesses various mechanisms that enable it to penetrate the human body. An effective and robust immune system can effectively combat harmful microorganisms, but, exposure to *Salmonella* can lead to infection and subsequent development of a systemic disease, impaired immune system, and susceptibility to illness due to factors such as poor diet, low hygiene, and immunosuppression (Murry, P.; Rosenthal, K. and Pfaller, 2013).

The aim of this study is isolation and molecular identification of *Gemella morbillorum* from mouth of patients that undergoes from typhoid infection with several complications.

Material and methods

1- Sample collection and bacterial cultivation

Of both sexes, 302 oral swabs were collected from patients suffering from typhoid fever in AL-Diwaniyah teaching hospital between September 2018 and June 2019. Each swab transferred to colleting tube containing transport media for temporary enhancement. Upon all specimens in the lab, streaked on routine and enrichment agar incubated in anaerobic jar supplements with gas bag Co_2 at 37C° over night for *Gemella morbillorum* isolation. Presumptive test for *Gemella* are gram staining and assessment hemolytic ability on Colombia

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agar 5% sheep blood (HiMedia, India)(Scola & Raoult, 1998). Biochemical tests catalase, cytochrome-oxidase, growth in 6.5% NaCl and Voges- Proskauer.

2- DNA extraction and PCR amplification

The presumptive isolates were introduced to molecular detection. DNA extraction performed according to company instructions (Geneaid, Taiwan). Primers designed by 3pluse software as forward: CGCGTAATACGTAGGTGGCA; Reverse ACATCTCACGACACGAGCTG for 16s rRNA gene detection. Gel electrophoresis visualized the product at 533bp under UV transilluminator.

3- Statistical analysis

SPSS was used to perform the statistical work to weather finding some differences at 0.05 of probability in X2 value measurement as mentioned in (Al-Ukaelii, S. A., & Al-Shaeb, 1998).

Results

1- Bacterial isolation and morphological identification

Total 302 swabs were screened for Gemella morbillorum from oral cavity of typhoid fever children's patients in both sexes ages (3-11). Data presented in table (1) showed the gram-positive bacteria that isolated by the culture media implicated and appearance of cocci cells arranged in pairs and sometimes in small chains as well as negative results for catalase and cytochrome oxidase in biochemical tests. Dominance of *Gemella morbillorum* within children significantly accounted for 22.18% (67 isolates).

Table (1): Gemella morbillorum Bacteria that Isolated from mouth children with Typhoid fever.

Gram Positive Bacteria	Total samples	Positive	Negative	%
Gemella morbillorum	**308**	**67**	**241**	**22.18**
X2	**1.03**			
P value	**0.05**			

2- Molecular confirmative test

16s rRNA specific regions provide confirmation for phenotypic identification specially in case of misdiagnosed species like Gemella morbillorum. After DNA extraction of Gemella morbillorum isolates which were 67 samples, the results of electrophoresis on agarose gel showed the 16S rRNA gene which is the diagnostic gene for *Gemella morbillorum* and the size of the gene 533bp as this was genetically proven for all isolates according to Figure (1).

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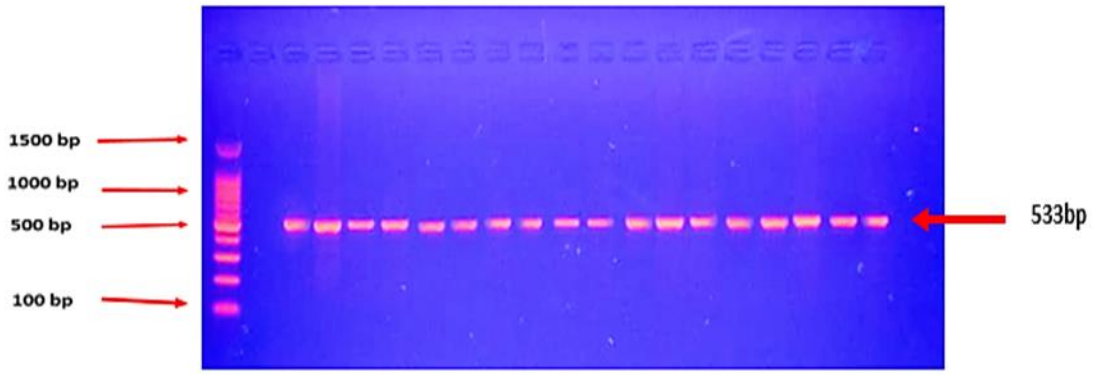

Figure (1): PCR product analysis of all isolates revealed the existence of 16s Ribosomal RNA gene which referred in lane (533 bp). While lane (100-1500) marker ladder.

Discussion

Even though *Gemella morbillorum* consider as commensal flora in many parts of human body ex: oral cavity, gut and urogenital tract (Lamont, 2018), but in certain circumstances could cause serious infection like endocarditis, septic shock, and periodontitis (Senent et al., 2008). Many topics described the morphological features of Gemella morbillorum as gram positive cocci, catalase and cytochrome oxidase additionally thrive in anaerobic conditions very well (Collins & Falsen, 2015). This species was mistaken as *streptococcus viridans* so that summitting molecular technique like PCR to confirm the identification (Parvataneni KC. et al., 2017). On basis 16s rRNA sequencing this bacteria were confirmed as *Gemella morbillorum* (Kilpper-Balz & Schleifer, 1988) which proved the genetic detection of this bacteria in current study. Since that event the researches focused on the prevalence of Gemella species in dental health, dental surgery, steroids therapy and diabetes (Benedetti et al., 2009). Important mechanism aid in pathogenetic of *Gemella morbillorum* through produce specific IgA1 protease which invade the adherence inhibitory activity of IgA in vitro consider as crucial virulence factor (Lomholt & Kilian, 2000). Another study revealed the genetic characterization of *Gemella morbillorum* as pathogenic causative agent empyema (Yamakawa et al., 2015). These reasons support current finding about increasing incidence of *Gemella morbillorum* in oral cavity simultaneously with other infections.

Conclusion

Biochemical tests uncertain the identification of Gemella morbillorum in many niches. Molecular methods using 16s rRNA certainly diagnosis the bacteria as causative agent in

patients suffering from immune disturbances or serious infections as typhoid fever especially in children.

References

[1] Akiyama, K., Taniyasu, N., Hirota, J., Iba, Y., & Maisawa, K. (2001). Recurrent aortic valve endocarditis caused by Gemella morbillorum: Report of a case and review of the literature. *Japanese Circulation Journal*, *65*(11), 997–1000. https://doi.org/10.1253/jcj.65.997

[2] Al-Ukaelii, S. A., & Al-Shaeb, S. M. (1998). *Statically Analysis by used SPSS program*. Al-Shoroq house for Publishers and advertisement Amaan, Jordan.

[3] Benedetti, P., Rassu, M., Branscombe, M., Sefton, A., & Pellizzer, G. (2009). Gemella morbillorum: An underestimated aetiology of central nervous system infection? *Journal of Medical Microbiology*, *58*(12), 1652–1656. https://doi.org/10.1099/jmm.0.013367-0

[4] Berger, U. (1992). The genus Gemella. In *The Prokaryotes* (2nd ed., pp. 1643–1653). pringer-Verlag New York NY.

[5] Brown, S. P., Cornforth, D. M., & Mideo, N. (2012). Evolution of virulence in opportunistic pathogens: Generalism, plasticity, and control. *Trends in Microbiology*, *20*(7), 336–342. https://doi.org/10.1016/j.tim.2012.04.005

[6] Christensen, J. J., & Ruoff, K. L. (2015). Aerococcus, Abiotrophia, and Other Aerobic Catalase-Negative, Gram-Positive Cocci. In *Manual of Clinical Microbiology* (pp. 422–436). John Wiley & Sons, Ltd. https://doi.org/https://doi.org/10.1128/9781555817381.ch24

[7] Collins, M. D., & Falsen, E. (2015). Gemella . *Bergey's Manual of Systematics of Archaea and Bacteria*, 1–8. https://doi.org/10.1002/9781118960608.gbm00578

[8] Dewhirst, F. E., Chen, T., Izard, J., Paster, B. J., Tanner, A. C. R., Yu, W. H., Lakshmanan, A., & Wade, W. G. (2010). The human oral microbiome. *Journal of Bacteriology*, *192*(19), 5002–5017. https://doi.org/10.1128/JB.00542-10

[9] Hooper, S. J., Crean, S., Fardy, M. J., Lewis, M. A. O., Spratt, D. A., Wade, W. G., & Wilson, M. J. (2007). *A molecular analysis of the bacteria present within oral squamous cell carcinoma Printed in Great Britain*. 1651–1659. https://doi.org/10.1099/jmm.0.46918-0

[10] Kilpper-Balz, R., & Schleifer, K. H. (1988). Transfer of Streptococcus morbillorum to the genus Gemella as Gemella morbillorum comb. nov. *International Journal of Systematic Bacteriology*, *38*(4), 442–443. https://doi.org/10.1099/00207713-38-4-442

[11] Lamont, R. J. (2018). The oral microbiota: dynamic communities and host interactions. *Nature Reviews Microbiology*. https://doi.org/10.1038/s41579-018-0089-x

[12] Lomholt, J. A., & Kilian, M. (2000). Immunoglobulin A1 protease activity in Gemella

haemolysans. *Journal of Clinical Microbiology*, *38*(7), 2760–2762. https://doi.org/10.1128/jcm.38.7.2760-2762.2000

[13] Lozupone, C., Cota-gomez, A., Palmer, B. E., Linderman, D. J., Charlson, E. S., Sodergren, E., Mitreva, M., Abubucker, S., Martin, J., Yao, G., Campbell, T. B., Flores, S. C., Ackerman, G., Stombaugh, J., Ursell, L., Beck, J. M., Curtis, J. L., Young, V. B., Lynch, S. V, … Fontenot, A. P. (n.d.). *Widespread Colonization of the Lung by Tropheryma whipplei in HIV Infection*. https://doi.org/10.1164/rccm.201211-2145OC

[14] Mitchell, R. G., & Teddy, P. J. (1985). Meningitis due to Gemella haemolysans after radiofrequency trigeminal rhizotomy. *Journal of Clinical Pathology*, *38*(5), 558–560.

[15] Murry, P.; Rosenthal, K. and Pfaller, M. (2013). *Medical Microbiology* (7th ed.). Elsevier. Philadelphia.

[16] Parvataneni KC., S, I., R, K., & LD., S. (2017). *Facklamia Species and Streptococcus pneumoniae Meningitis: A Case Report and Review of the Literature. 2*(September), 2633851. https://doi.org/10.1093/o

[17] Petrova, M. I., Balzarini, J., Vanderleyden, J., & Lebeer, S. (2013). Vaginal microbiota and its role in HIV transmission and infection. *FESM Microbial Rev.*, *37*(5), 762–792. https://doi.org/10.1111/1574-6976.12029

[18] Pushalkar, S., Ji, X., Li, Y., Estilo, C., Yegnanarayana, R., & Singh, B. (2012). *Comparison of oral microbiota in tumor and non-tumor tissues of patients with oral squamous cell carcinoma*.

[19] Round, J. L., & Mazmanian, S. K. (2009). *The gut microbiota shapes intestinal and disease*. *9*(MAy), 313–324. https://doi.org/10.1038/nri2515

[20] Sawada, A., Mochizuki, K., Katada, T., Kawakami, H., Yamamoto, T., Mikamo, H., & Watanabe, K. (2009). Gemella species-associated late-onset endophthalmitis after trabeculectomy with adjunctive mitomycin c. *Journal of Glaucoma*, *18*(6), 496–497. https://doi.org/10.1097/IJG.0b013e31818c6f07

[21] Scola, B. L. A., & Raoult, D. (1998). *Molecular Identification of Gemella Species from Three Patients with Endocarditis*. *36*(4), 866–871.

[22] Senent, C., Sancho, J. N., Chiner, E., Signes-Costa, J., Camarasa, A., & Andreu, A. L. (2008). [Pleural empyema caused by Gemella species: a rare condition]. *Archivos de Bronconeumologia*, *44*(10), 574—577. https://doi.org/10.1157/13126839

[23] Shulzhenko, N., Morgun, A., Hsiao, W., Battle, M., Yao, M., Gavrilova, O., Orandle, M., Mayer, L., Macpherson, A. J., Mccoy, K. D., Fraser-liggett, C., & Matzinger, P. (2011). Crosstalk between B lymphocytes , microbiota and the intestinal epithelium governs immunity versus metabolism in the gut. *Nature Medicine*, *17*(12), 1585–1593.

https://doi.org/10.1038/nm.2505

[24] Takayasu, L., Suda, W., Takanashi, K., Iioka, E., Kurokawa, R., Shindo, C., Hattori, Y., Yamashita, N., Nishijima, S., Oshima, K., & Hattori, M. (2017). Circadian oscillations of microbial and functional composition in the human salivary microbiome. *DNA Research*, *24*(3), 261–270. https://doi.org/10.1093/dnares/dsx001

[25] Van De Wijgert, J. H. H. M., Borgdorff, H., Verhelst, R., Crucitti, T., Francis, S., Verstraelen, H., & Jespers, V. (2014). The vaginal microbiota: What have we learned after a decade of molecular characterization? *PLoS ONE*, *9*(8). https://doi.org/10.1371/journal.pone.0105998

[26] Vasishtha, S., Isenberg, H. D., & Sood, S. K. (1996). Gemella morbillorum asa Cause of Septic Shock. *Clinical Infectious Diseases*, *22*(6), 1084–1086.

[27] Yamakawa, H., Hayashi, M., Tanaka, K., & Kuwano, K. (2015). Empyema due to gemella morbillorum is diagnosed by 16s ribosomal RNA gene sequencing and a phylogenetic tree analysis: A case report and literature review. *Internal Medicine*, *54*(17), 2231–2234. https://doi.org/10.2169/internalmedicine.54.4950