ANTIBIOTIC SUSCEPTIBILITY PATTERN OF OPTOCHIN-RESISTANT VIRIDANS STREPTOCOCCI IN SPUTUM SAMPLES OF PATIENTS IN CENTRAL HOSPITAL, WARRI, DELTA STATE.

Okoye E.L[1], Nnanna J.C.[2], Esimone C.O.[2], Ikeagbunam M.N.[2]

[1]Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka

[2]Department of Pharmaceutical Microbiology and Biotechnology, Nnamdi Azikiwe University, Awka.

ABSTRACT

The viridans group streptococci (VGS), are a heterogeneous group of bacteria belonging to the genus *Streptococcus*. They act as a reservoir for antibiotic resistance genes, thus transferring different resistance traits to more pathogenic organisms like *S. pneumonae* and *S. pyogenes*. The aim of this study was to evaluate the antibiotic resistance in optochin-resistant (VGS) from the sputum samples of patients from all age groups at Central Hospital, Warri, Delta State, Nigeria. A total of 200 sputum samples were collected from patients with clinical symptoms of tuberculosis, pneumonia and other respiratory tract infections. The VGS were isolated and identified using standard methods. The antibiotic susceptibility testing of the isolated VGS was performed according to Clinical and Laboratory Standards Institute's recommendations. A total of 48 (24 %) VGS were isolated. All of the isolates (100 %) were susceptible to imipenem and resistant to cefotaxime. 95.83 % of the isolates were susceptible to vancomycin while 89.58 %, 83.33 %, 79.16 %, 77.08 %, 68.75% and 52.08 % of the isolates were resistant to penicillin, trimethoprim-sulphamethoxazole, tetracycline, amoxicillin, clindamycin and levofloxacin respectively. The isolated VGS were 22(45.83 %) resistant and 11(22.90 %) intermediately resistant to erythromycin. Multidrug resistance was seen in all the VGS isolated, with co-resistance to β-lactam, macrolides, tetracycline and trimethoprim-sulphamethoxazole. This study showed that imipenem, vancomycin and clindamycin remain the drugs of choice for the treatment of infections caused by VGS. Multidrug resistance, especially to β-lactam antibiotics was observed in the VGS isolated. There is need for continuous evaluation of antimicrobial susceptibility pattern among VGS for effective antibiotic therapy and to track resistance overtime.

Keywords: viridans group streptococci, antibiotics resistance, β-lactam, multidrug resistant.

INTRODUCTION: The viridans group streptococci (VGS), are a heterogeneous group bacteria belonging to the genus *Streptococcus*. They are Gram-positive cocci, catalase-negative, leucine aminopeptidase positive and pyrrolidonylaryl amidase negative (Desai *et al.*, 2017). They can be differentiated from *S. pneumoniae* by their optochin resistance and bile insolubility, although *S. pneumoniae* isolates resistant to optochin have been reported in different geographical regions (Wisiva *et al*., 2021). VGS are part of the normal microbiota of the oral cavity as well as the upper respiratory, gastrointestinal and female genital tracts. The term 'viridans' (from the

Latin word "viridis", which means green) refers to the green discolouration on blood agar produced by alpha haemolysis and is characteristic of this group (Wisiva *et al.*, 2021; Park *et al.,* 2015). Owing to their variability and overlap of their microbial characteristics, classification of VGS has posed a challenge (Wisiva *et al.*, 2021; Guerrero *et al.*, 2018). Currently, VGS are classified into six major groups namely: *S. mitis, S. mutans, S. salivarius, S. sanguinis, S. anginosus* and *S. bovis* (Teles *et al.*, 2011; Nagata *et al.*, 2012; Park *et al.,* 2015). VGS can cause a number of important clinical syndromes, though regarded as low virulence bacteria (Park *et al.,* 2015). VGS are known to be the causative agent in infective endocarditis (Sejong-Chun *et al.*, 2015) and dental caries (Shree *et al.*, 2016) They are as well, associated with bacteraemia and systemic sepsis in neutropenic patients and preterm infants (Park *et al.,* 2015; Sejong-Chun *et al.*, 2015) . They have also been implicated occasionally in meningitis, usually in patients with head or neck structural abnormalities, or pneumonia (Park *et al.*, 2015) . In addition, they act as a reservoir for antibiotic resistance genes, transferring different resistance traits to more pathogenic organisms like *S. pneumoniae* and *S. pyogenes* (Sappala *et al.,* 2003; Guerrero *et al.*, 2018). Previously, VGS were susceptible to β-lactam antimicrobial agents, aminoglycosides, tetracyclines, and macrolides (Wisiva *et al.,* 2021), however, antibiotic resistance in VGS, particularly to penicillin and macrolides, has recently been reported globally (Park *et al.,* 2015; Chitra *et al.,* 2015; *Prakash et al.*, 2015; Rotimi *et al.,* 2005*;* Matsuda *et al.,* 2012; Nemoto *et al.*, 2011), posing a growing health concern. There is need for continuous evaluation of antimicrobial susceptibility among VGS, in order to develop an appropriate antibiotic therapy and treatment options for the different infections caused by VGS. There is limited data on the antibiotic susceptibility pattern of VGS isolated from sputum samples. Hence, in this study, we aimed to evaluate the antibiotic resistant pattern of VGS isolated from sputum samples of adults with different clinical symptoms, in Central Hospital, Warri, Delta State, Nigeria.

MATERIALS AND METHOD

STUDY AREA, DESIGN, AND PERIOD: this study was a hospital-based prospective study and was conducted between June to December, 2020, in Central Hospital, Warri, Delta State. Informed consent forms were given to all subjects recruited in this study before sample collection. Sputum samples were collected from patients (all age groups) with clinical symptoms of tuberculosis, pneumonia and other respiratory tract infections. Healthy patients and patients that received antibiotics within the last 4 weeks were excluded from this study. This study was approved by the Ethics and Research Committee, Delta State Hospitals Management Board, Warri Medical Zone, Central Hospital, Warri. The clearance certificate number is CHW/ECC VOL 1/174.

SAMPLE COLLECTION AND IDENTIFICATION: a total of 200 sputum samples were collected and inoculated onto 5 % sheep blood agar plates. The inoculated plates were incubated at 37°C in 5% CO_2 for 18–24h. The isolates were identified using alpha-hemolysis, gram stain, catalase test and optochin susceptibility test.

ANTIMICROBIAL SUSCEPTIBILITY TESTING: this was performed using the disk diffusion method according to the

guidelines by the Clinical and Laboratory Standard Institute (CLSI, 2019). Ten antibiotics (Oxoid, UK) including erythromycin 15g), clindamycin (2g), tetracycline, vancomycin (30g), levofloxacin (5g), amoxicillin (10g), penicillin (10iu), cefotaxime (30g), imipenem (10g) and trimethoprim-sulpha methoxazole (10g), were tested against the isolated VGS. A bacterial suspension of 0.5 McFarland standard for each of the isolates was inoculated onto Mueller Hinton agar plates, supplemented with 5 % sheep blood. Afterwards, the disks were placed onto the plates and incubated at 37°C in 5% CO_2 for 18–24h. The results were interpreted as susceptible, intermediate and resistant based on the breakpoints given by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2020).

STATISTICAL ANALYSIS: comparison of the susceptibility rates of the viridians group of streptococci were performed by chi-square test. Statistical significance was defined as $P<0.05$.

RESULTS: a total of 48 (24 %) VGS were isolated based on optochin resistance (Tables 1 and 2). All of the isolates (100%) were susceptible to imipenem and resistant to cefotaxime. 95.83 % and 10.42 % of the isolates were susceptible and resistant to vancomycin respectively. 89.58 % and 77.08 % of the isolates were resistant to Penicillin and amoxicillin respectively. Resistance to penicillin, amoxicillin and cefotaxime representing the β-lactams was found to be 32(72.91 %). 11(22.90 %) and 22(45.83 %) were intermediately and resistant to erythromycin while 15(31.25 %) were resistant to clindamycin. Isolates resistant to both erythromycin and clindamycin comprised of 33.33 %. Resistance to erythromycin was found in 18(41.86 %) of the penicillin non-susceptible isolates and 21(55.26 %) of tetracycline resistant isolates. 37. 5 % of the isolates were resistant to both erythromycin and penicillin. Resistance to tetracycline was found in 38 (77.16%) of all the isolates. Tetracycline resistance was found in 37 (86.05%) of the penicillin-non-susceptible isolates. 43.75 % were resistant to both erythromycin and tetracycline, while 77.08 % were resistant both tetracycline and penicillin. The resistance of the isolates to levofloxacin was 52.08 %. All the isolates were resistant to more than three of the antibiotics (Tables 1 and 2).

Table 1: Antibiotic Susceptibility Test of the isolated VGS

ISOLATES	**E 15µg**		**DA 2µg**		**LEV 5µg**		**TET 30µg**		**SXT 10µg**		**IPM 10µg**		**P 10iu**		**AML 10µg**		**VA 30µg**		**CTX 30µg**		**OPT**	
	INHIBITION ZONE DIAMETER (mm)																					
SP59	28	**S**	25	**S**	20	S	10	**R**	15	**S**	31	**S**	00	R	00	R	20	**S**	00	R	00	R
SP43	19	**I**	28	**S**	24	**S**	10	**R**	00	R	35	**S**	00	R	00	R	20	**S**	00	R	00	R
SP62	00	R	00	R	22	**S**	00	R	00	R	36	**S**	00	R	00	R	20	**S**	00	R	00	R

SP86	29	**S**	29	**S**	26	**S**	25	**S**	00	R	36	**S**	00	R	00	R	22	**S**	00	R	00	R
SP93	19	**I**	28	**S**	25	**S**	00	R	00	R	35	**S**	00	R	00	R	25	**S**	00	R	00	R
SP40	20	**I**	35	**S**	00	R	25	**S**	00	R	35	**S**	00	R	00	R	20	**S**	00	R	00	R
SP1	27	**S**	25	**S**	24	**S**	10	**R**	00	R	38	**S**	00	R	15	**R**	20	**S**	00	R	00	R
SP35	16	**R**	29	**S**	20	**S**	00	R	00	R	38	**S**	00	R	00	R	20	**S**	00	R	00	R
SP12	00	R	00	R	00	R	00	R	00	R	38	**S**	00	R	00	R	20	**S**	00	R	00	R
SP49	25	**S**	35	**S**	00	R	05	R	21	**S**	30	**S**	00	R	20	**S**	23	**S**	00	R	00	R
SP28	21	**I**	31	**S**	22	**S**	00	R	00	R	38	**S**	00	R	00	R	22	**S**	00	R	00	R
SP90	20	**I**	30	**S**	00	R	22	**I**	15	**S**	36	**S**	00	R	12	**R**	21	**S**	00	R	00	R
SP86	33	**S**	30	**S**	22	**S**	21	**I**	00	R	40	**S**	00	R	00	R	21	**S**	00	R	00	R
SP106	15	**R**	25	**S**	00	**I**	00	**I**	00	R	20	**S**	00	R	00	R	20	**S**	00	R	00	R
SP104	20	**I**	29	**S**	00	R	00	R	00	R	37	**S**	00	R	00	R	21	**S**	00	R	00	R
SP105	17	**R**	29	**S**	00	R	00	R	00	R	38	**S**	00	R	00	R	20	**S**	00	R	00	R
SP101	19	**I**	30	**S**	00	R	00	R	09	**R**	36	**S**	00	R	00	R	20	**S**	00	R	00	R
SP100	30	**S**	30	**S**	22	**S**	02	R	00	R	42	**S**	00	R	13	**R**	20	**S**	00	R	00	R
SP60	28	**S**	26	**S**	00	R	00	R	00	R	34	**S**	00	R	00	R	20	**S**	00	R	00	R
SP70	29	**S**	27	**S**	20	**S**	00	R	16	**S**	40	**S**	00	R	11	**R**	20	**S**	00	R	00	R
SP77	25	**S**	28	**S**	20	**S**	00	R	11	**R**	35	**S**	00	R	10	**R**	20	S	00	R	00	R
SP74	17	**R**	26	**S**	15	**R**	00	R	00	R	39	**S**	00	R	11	**R**	20	**S**	00	R	00	R
SP52	15	**R**	18	**R**	10	**R**	15	**S**	00	R	40	**S**	00	R	11	**R**	15	**R**	15	**R**	00	R
SP61	15	**R**	30	**S**	24	**S**	25	**S**	00	R	38	**S**	00	R	00	R	20	**S**	00	R	00	R

SP65	35	**S**	34	**S**	20	**S**	00	R	05	R	40	**S**	00	R	10	**R**	20	**S**	00	R	00	R
SP81	30	**S**	26	**S**	20	**S**	27	**S**	10	**R**	38	**S**	00	R	10	R	20	**S**	00	R	00	R
SP111	00	R	00	R	25	S	14	**R**	15	**S**	39	**S**	00	R	00	R	20	**S**	00	R	00	R
SP103	30	**S**	27	**S**	15	**R**	29	**S**	00	R	30	**S**	18	**S**	18	R	20	**S**	00	R	00	R
SP91	12	**R**	13	**R**	00	**R**	16	**R**	00	R	25	**R**	15	**R**	16	**R**	16	**S**	13	R	00	R
SP5	12	**R**	00	R	00	R	11	**R**	00	R	27	**R**	15	**R**	12	**R**	15	**R**	00	R	00	R
SP71	15	**R**	14	**R**	00	R	12	**R**	05	R	25	**S**	18	**S**	20	**I**	18	**S**	16	**R**	00	R
SP72	00	R	00	R	00	R	00	R	05	R	25	**S**	18	**S**	20	**I**	20	**S**	00	R	00	R
SP132	00	R	00	R	00	R	15	**R**	00	R	36	**S**	20	**S**	20	**I**	20	**S**	10	R	00	R
SP133	00	R	00	R	00	R	15	**R**	00	R	35	**S**	00	R	10	**R**	20	**S**	00	R	00	R
SP124	00	R	00	R	00	R	10	R	00	R	30	**S**	10	R	15	**R**	20	**S**	00	R	00	R
SP119	20	**I**	30	**S**	00	R	15	**R**	00	R	35	**S**	00	R	10	R	20	**S**	00	R	00	R
SP134	15	**R**	25	S	20	S	15	**R**	00	R	38	**S**	15	**R**	20	**I**	21	**S**	10	R	00	R
SP127	00	R	00	R	00	R	15	**R**	09	R	35	**S**	15	**R**	19	R	20	**S**	10	R	00	R
SP128	21	**I**	27	**S**	00	R	10	R	18	**S**	32	**S**	00	R	00	R	21	**S**	00	R	00	R
SP130	17	**R**	29	**S**	25	**S**	00	R	00	R	38	**S**	00	R	17	**R**	20	S	00	R	00	R
SP131	00	R	00	R	00	R	10	R	00	**R**	30	**S**	00	R	00	R	18	**S**	00	R	00	R
SP123	25	**S**	20	**S**	23	**S**	12	R	15	**S**	32	**S**	12	**R**	15	**R**	19	**S**	12	R	00	R
SP146	25	**S**	30	**S**	15	**R**	10	R	00	R	34	**S**	10	R	19	**I**	20	**S**	00	R	00	R
SP145	20	**I**	25	**S**	10	**R**	14	**R**	00	R	33	**S**	12	R	20	**I**	20	**S**	10	R	00	R
SP160	30	**S**	30	**S**	25	**S**	11	R	00	R	42	**S**	15	**R**	20	**I**	20	**S**	12	R	00	R

SP140	21	**I**	20	**S**	00	R	15	**S**	00	R	40	S	14	**R**	22	**S**	20	**S**	10	R	00	R
SP154	00	R	00	R	21	**S**	09	R	15	**S**	40	**S**	18	**S**	19	**I**	20	**S**	10	**R**	00	R
SP148	00	R	00	R	24	**S**	11	**R**	10	R	40	**S**	17	**R**	21	**I**	21	**S**	10	R	00	R

KEY: Erythromycin (E); Clindamycin (DA); Levofloxacin (LEV); Tetracycline (TET); Imipenem (IPM); Penicillin (P); Amoxycillin (AML); Trimethoprim-sulfamethoxazole (SXT); Vancomycin (VA); Cefotaxime (CTX); Susceptible (S); Intermediate (I); Resistant (R), international unit (iu).

Table 2: Number of VGS Susceptible, Intermediate and Resistant

Antibiotics	**Sensitive N (%)**	**Intermediate N (%)**	**Resistant N (%)**
Erythromycin	15 (31.25 %)	11 (22.90 %)	22 (45.83 %)
Clindamycin	33 (68.75%)	00 (00 %)	15 (31.25 %)s
Levofloxacin	22 (45.80 %)	1 (2.08 %)	25 (52.08 %)
Tetracycline	7 (14.58 %)	3 (6.25 %)	38 (79.16 %)
Trimthoprim-sulphamethoxazole	8 (16.67 %)	00 (00 %)	40 (83.33 %)
Imipenem	48 (100 %)	00 (00%)	00 (00 %)
Penicillin	5 (10.42 %)	00 (00 %)	43 (89.58 %)
Amoxycillin	2 (4.17 %)	9 (18.75 %)	37 (77.08 %)
Vancomycin	46 (95.83 %)	00 (00 %)	2 (4.16 %)
Cefotaxime	00 (00%)	00 (00 %)	48 (100 %)

DISCUSSION: there is an alarming rate of antibiotics resistance among VGS. In our study, there was a high rate (89. 58%)of resistance in the VGS to penicillin. Penicillin-resistant VGS remain the genetic reservoir for β lactam resistance in some pathogenic organisms such as *S. pneumoniae*]. Andrea *et al.,* (2009) reported an unusually high level resistance to β-lactam with high MIC values. High rates of penicillin resistance (40.7 % to 70 %) has also been reported from the rest of the world (Huang *et al.,* 2007; Andrea *et al.,* 2009). This could be as a result of the presence of mosaic penicillin binding protein (PBP) genes that encodes for PBPs with reduced affinity to β- lactam (Amoroso *et al.,* 2001)

The high resistance of VGS to amoxicillin (77.08 %) observed in this study contrasts with the findings of Morva *et al.,* (2021), who found that oral VGS isolated from children at risk of infective endocarditis had a high susceptibility to amoxicillin (90.2 %). Similar studies have also been reported on VGS susceptibility to amoxicillin (Chundiri *et al.,* 2012; William *et al.,* 2012). This present work is also in line with the work of Matsuda *et al.,* (2012) where oral VGS from healthy Japanese children and adults were highly resistant to amoxicillin.

It was observed in this work, that 45.83% of the isolates were resistant to erythromycin. In a study from Japan, on VGS from normal Flora of healthy children and healthy adults, 55.2% and 40.5% respectively, of the isolates were resistant to erythromycin. In another study in healthy Greek children, 38.5% of the isolates were erythromycin resistant (Ioannidou *et al.*, 2001). A study in France, also reported resistance rates of 41 % - 53 % to erythromycin in VGS (Bryskier *et al.*, 2002). It has been demonstrated that macrolide resistant VGS is associated with co-resistance to tetracycline (Malhotra-Kumar *et al.*, 2004) and this correlate with our study, in which (55.26 %) of the erythromycin resistant VGS were also resistant to tetracycline. 10.42 % and (52.08 %) of VGS isolates in this work, were resistant to vancomycin and levofloxacin respectively. Rawan *et* al., (2015) reported a resistance rate of 4 % in VGS to vancomycin and 9 % to levofloxacin. The VGS were isolated from the oral cavity of patients in Jordan. This work contradicts the work of Sappala *et al.*, (2003), who reported 100 % susceptibility to vancomycin and 90 % to levofloxacin, in VGS isolated from normal flora in adult patients in Finland. It has been previously documented that the susceptibility of VGS to glycopeptide antibiotics like vancomycin has remained high (Tuohy and Washington, 1997; Mcwhinney *et al.*, 1993). However, there is a need for periodic assessment of antimicrobial activity to determine change in susceptibility patterns of this organism.

Furthermore, 83.33 % of the VGS isolates in this present study were resistant to trimethoprim-sulphamethoxazole. This correlates with the study of Wil´en *et al.*, (2009), that reported a 100 % resistance to trimethoprim-sulphamethoxazole in VGS isolated from throat flora in Kampala, Uganda. Buwembo *et al.*, (2012) reported a low level resistance to trimethoprim-sulphamethoxazole (15.5%) in VGS isolates from oral flora found in Uganda. Multidrug resistance (MDR) was observed in 100 % of the VGS isolated in this study. The most common pattern of MDR seen in this present study, was co-resistance with β-lactam, macrolide, tetracycline and trimethoprim-sulphamethoxazole. The rate of MDR in VGS observed in this study was higher than that reported by Dhotre *et al.*, (2015), who reported that 11% of VGS isolates from patients undergoing tooth extraction in India were resistant to as many as eight antimicrobials. William *et al.*, (2012) also showed in their study that 14% of the VGS isolates were resistant to more than two antibiotics. Morva *et al.*, (2021), reported MDR rate of 7.8% in oral VGS from children at risk of infective endocarditis.

CONCLUSIONS: this study has confirmed the growing rate of VGS to β-lactam antibiotics and the emerging multidrug resistance VGS that might serve as a reservoir for resistance traits, that could be transferred to more pathogenic organisms. Co-resistance with a number of antibiotics were also observed. Imipenem, vancomycin and clindamycin remains the drug of choice for the treatment of infections caused by this group of organism.

AUTHOR STATEMENT: this work is carried out in conjunction with all the authors. The authors participated equally in the design, literature review, analysis and in the writing of the manuscript. The manuscript was read and approved by all the authors.

CONFLICT OF INTEREST: The authors declare no conflict of interest.

REFERENCES

Abu-zineh R., Dar-Odeh N. and Shehabi A. (2015). Macrolide resistance genes and virulence factors of common viridans Streptococci species colonizing oral cavities of patients in Jordan. Oral Health Dent Manag 2015;14:337-41

Amoroso A, Demares D., Mollerach, M. *et al*., (2001). All detectable high-molecular-mass penicillin-binding proteins are modified in a high-level β-lactam-resistant clinical isolate of Streptococcus mitis . Antimicrobial Agents and Chemotherapy 45, 2075–81

Andrea K, Ralf RR and Regine H (2009). Streptococcus mitis with unusually high level resistance to β-lactam antibiotics. Microbial Drug resistnace, vol 4 No 1. https://doi.org/10.1089/mdr.1998.4.45

Buwembo W, Charles MR., G¨ote S and Fred K (2012). Cotrimoxazole Prophylaxis Specifically Selects for Cotrimoxazole Resistance in Streptococcus mutans and Streptococcus sobrinus withVaried Polymorphisms in the Target Genes folA and folP. International Journal of Microbiology Volume 2012, Article ID 916129, 10 pages doi:10.1155/2012/916129

Chitra N, Mangayarkarasi V, Balakrishnan D and Smiline Girija A.S. (2015). Resistance Patterns of Viridans Group of Streptococci isolated in transient bacteremia after third molar surgery. Int J Curr Microbiol App Sci 2015;4(6):78-85

Clinical and Laboratory Standard Institute (2019). Performance Standard for Antimicrobial Susceptibility Testing. Approved Standard M 100-S24. Wayne, PA: Clinical and Laboratory Standard Institute (CLSI).

Chunduri NS, Madasu K, Goteki VR, *et al*., (2012). Evaluation of bacterial spectrum of orofacial infections and their antibiotic susceptibility. Ann Maxillofac Surg 2012; 2(1):46-50. https://doi.org/10.4103/2231-0746.95318

Desai N, Steenbergen J, and Katz DE (2017). Antibiotic Resistance of Non-pneumococcal Streptococci and Its Clinical Impact. Springer International Publishing AG 2017 D.L. Mayers et al. (eds.), Antimicrobial Drug Resistance, DOI 10.1007/978-3-319-47266-9_2

Dhotre S, Dharne M, Potdar S, *et al*., (2015). Viridians Group Streptococci in Patients Undergoing Tooth Extraction. Eur J Gen Med 2015;12(3):227-33. https://doi.org/10.15197/ejgm.01406

Guerrero-Del-Cueto F, Ibanes-Guti´errez C, Vela´zquez CA *et al*., (2018). "Microbiology and clinical characteristics of viridans group streptococci in patients with cancer," The Brazilian Journal of Infectious Diseases, vol. 22, no. 4, pp. 323–327, 2018

Han S, Bae E, Lees J, *et al*., (2013). Clinical characteristics and antibiotic susceptibility of viridans streptococcal bacteraemia in children with febrile neutropenia. Infection. 2013;41:917–924.

Huang WT, Chang LY, Hsueh PR, *et al*., (2007). Clinical features and complications

of Viridans Streptococci bloodstream infection in pediatric hemato-oncology patients. J Microbiol Immunol Infect. 2007;40:349–354

Ioannidou S, Tassios PT, Kotsovili-Tseleni A *et al*., (2001). Antibiotic resistance rates and macrolide resistance phenotypes of viridans

Malhotra-Kumar S, Lammens C, Martel A ,*et al*.., (2004). "Oropharyngeal carriage of macrolide-resistant viridans group streptococci: a prevalence study among healthy adults in Belgium. Journal of Antimicrobial Chemotherapy, 2004; 53: 271–276

Matsuda K., Nemoto H., Nakano K., Naka S., Nomura R., Ooshima T. (2012). Amoxicillin-Resistant Oral Streptococci identified in Dental Plaque Specimens from Healthy Japanese Adults. Journal of Cardiology (2012)59, 285-290. Available on www.sciencedirect.com

McWhinney PHM, Patel S, Whiley RA, Hardie JM, Gillespie SH and Kibbler CC (1993). Activities of potential therapeutic and prophylactic antibiotics against blood culture isolates of viridans group streptococci from neutropenic patients receiving ciprofloxacin. Antimicrob Agents Chemother 1993; 37(11): 2493-95.

Morva M, Nilgoon P, Kazem B., Zahra S and Farahnaz S (2021). Antimicrobial Susceptibility Pattern of Oral Viridans Group Streptococci in Children at Risk of Infective Endocarditis. Acta facultatis medicae Naissensis 2021; 38(2): 156-163 UDC: 579.862:616.126-002-053.2. DOI: 10.5937/afmnai38-28448

Nagata M, Ueda O, Shobuike T, Muratani T, Aoki Y, and Miyamoto H (2012). "Emergence of optochin-resistance among Streptococcus pneumonia in Japan,"Open Journal of Medical Microbiology, vol. 2, no. 1, pp. 8–15, 2012.

Nemoto K., Nakano K., Masuda K., *et al*., (2011). Distribution of oral streptococci highly resistant to amoxicillin in dental plaque specimens from Japanese children and adolescents. Journal of Medical Microbiology. 2011; 60: 1853-1859.

Park T., Barrett L. and Jones N. (2015). Invasive streptococcal disease: a review for clinicians. BritishMedicalBulletin,2015,115:77–89.doi:10.1093/bmb/ldv027. https://academic.oup.com/bmb/article/115/1/77/260207 by guest on 03 December 2021

Prakash D, Ramesh K, Gopinath N, *et al*., (2015). Antibacterial efficacy of Syzygium aromaticum extracts on multi-drug resistant Streptococcus mutans isolated from dental plaque samples. J Biochem Technol 2014;3(5):155-7

Rawan FA, Najla SD and Asem AS (2015). Macrolide Resistance Genes and Virulence Factors of Common Viridans Streptococci Species Colonizing Oral Cavities of Patients in Jordan. Oral Health and Dental Management · December 2015 DOI: 10.4172/2247-2452.1000839 https://www.researchgate.net/publication/284442697

Rotimi V., Salako N, Mokaddas E, et al., (2005). High frequency of isolation of

antibiotic-resistant oral Viridans streptococci from children in Kuwait. J Chemother 2005; 17(5):493-501. https://doi.org/10.1179/joc.2005.17.5.493

Sappala H, Marjo H, Mohammad Al-Juhaish, Helinä J, Jari J and Pentti H (2003). Antimicrobial susceptibility patterns and macrolide resistance genes of viridans group streptococci from normal flora. Journal of Antimicrobial Chemotherapy (2003) 52, 636–644 DOI: 10.1093/jac/dkg423

Sejong Chun MD, Hee Jae Huh MD, and Nam Yong Lee MD (2015). Species-Specific Difference in Antimicrobial Susceptibility Among Viridans Group Streptococci. Ann Lab Med 2015;35:205-211
http://dx.doi.org/10.3343/alm.2015.35.2.205

Shree VD, Vilas LJ, Milind SD, Basavraj SN (2016). Susceptibility, Resistance and Treatment Strategy for Infections Caused by Viridans Group Streptococci - A Review. Journal of Krishna Institute of Medical Sciences University. Vol. 5, No. 4, October-December 2016. ISSN 2231-4261

Teles C, Smith A, Ramage G, and Lang S (2011). "Identification of clinically relevant viridans group streptococci by phenotypic and genotypic analysis,"European Journal of Clinical Microbiology & Infectious Diseases, vol. 30, no. 2, pp. 243–250, 2011

The European Committee on Antimicrobial Susceptibility Testing (2020). Breakpoint tables for interpretation of MICs and Zone diameters, Version 10.0. http://www.eucast.org/clinical_breakpoints/

Tuohy M and Washington JA (1997). Antimicrobial susceptibility of Viridans streptococci. Diagn Microbiol Infect Dis 1997; 29(4): 277-80

William B, Rwenyonyi CM, Swedberg G, Kironde F (2012). Cotrimoxazole prophylaxis specifically selects for cotrimoxazole resistance in Streptococcus mutans and Streptococcus sobrinus with varied polymorphisms in the target genes folA and folP. Int J Microbiol 2012;2012:916129.
https://doi.org/10.1155/2012/916129

Wil´en M, Buwembo W, Sendagire H, Kironde F, and Swedberg G. (2009). "Cotrimoxazole resistance of Streptococcus pneumoniae and commensal streptococci from Kampala, Uganda," Scandinavian Journal of Infectious Diseases, vol. 41, no. 2, pp. 113–121, 2009.

Wisiva T P, Nurma S S, Miftahuddin M K, Wisnu T, Wahyu F S and Dodi S (2021). Isolation and Identification of Optochin-Resistant Viridans Group Streptococci from the Sputum Samples of Adult Patientsin Jakarta, Indonesia. Hindawi International Journal of Microbiology Volume 2021. https://doi.org/10.1155/2021/6646925