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Antibiotic Susceptibility Patterns of Gram Positive Bacteria (*Streptococcus viridans* and *Enterococcus faecalis*) Isolated Clinically from Patients in El Jalaa Hospital for Surgery and Accidents in Benghazi City – Libya

[1]Dareen El shareef Jadullah [2]Noor alhooda M. Alawkally.
[1]Microbiology, Faculty of pharmacy, Qurina International University, Benghazi– Libya,
[2] Medical laboratory Department, Higher Institute of Science and Technology, Suluq, Libya.
[1] Email: dareenelshareef@qiu.edu.ly
[2]E-mail: noornooor1973@gmail.com

Abstract— ***Background: Streptococcus viridans bacteria and Enterococcus faecalis bacteria are considered gram positive and caused several diseases and the most famous of them is like infectious endocarditis. Objectives: To evaluate antibiotic resistance trends in Streptococcus Viridans and Enterococcus faecalis over time. Materials and Methods: This cross-sectional study was conducted on a group of 100 (69 males, 31 females) suspected of having gram-positive bacteria & in the age range of 14 days to over 46 years old in Al-Jalaa Hospital for surgery & accidents (Benghazi) from January to March in 2022. Using standard isolation and identification procedures a total of 100 isolates were obtained from patients. The isolated samples are 60 urine samples,3 stool samples and 27 swab samples and 10 semen samples. The antibiotics were used during the study period for all specimens were the antibiotics that demonstrated in-vitro activity regularly against all culture organisms. Results: Our study shows that Streptococcus viridans may be resistant to Amoxicillin and Cefuroxime & more sensitive to Ciprofloxacin and Levofloxacin whereas Enterococcus faecalis are more resistant to Amoxicillin and Sulfamethoxazole & more sensitive to levofloxacin. Conclusions: Susceptibility rates for some relevant antibiotics declined for clinically isolated bacteria. This worrisome trend highlights the need to improve antimicrobial stewardship efforts to limit unnecessary antibiotic use and preserve empirical treatment options.***

Keywords- ***Antibiotic, Gram positive bacteria , Hospitalized patients.***

I. INTRODUCTION

A. Types of bacteria:

A.1 Enterococcus faecalis:

Enterococcus faecalis formerly classified as part of the group D Streptococcus system is a Gram-positive, commensal bacterium inhabiting the gastrointestinal tracts of humans.[1][2] Like other species in the genus Enterococcus, E. faecalis is found in healthy humans and can be used as a probiotic. As an opportunistic pathogen, E. faecalis can cause life-threatening infections, especially in the nosocomial (hospital) environment, where the naturally high levels of antibiotic resistance found in E. faecalis contribute to its pathogenicity.[2] E. faecalis has been frequently found in reinfected, root canal-treated teeth in prevalence values ranging from 30% to 90% of the cases.[3] Re-infected root canal-treated teeth are about nine times more likely to harbor E. faecalis than cases of primary infections.[4]

A.2 Streptococcus viridans:

The viridans streptococci are a large group of commensal streptococcal Gram-positive bacteria species that are α-

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hemolytic, producing a green coloration on , although some species in this group are actually γ-hemolytic, meaning they produce no change on blood agar.[5] The pseudo-taxonomic term "Streptococcus viridans" is often used to refer to this group of species, but writers who do not like to use the pseudo taxonomic term (which treats a group of species as if they were one species) prefer the terms viridans streptococci,[6] viridans group streptococci (VGS), or viridans streptococcal species.

B. Diseases:

B.2 Enterococcus faecalis :

Enterococcus faecalis (E. faecalis) is a species of bacteria that lives harmlessly in the digestive tract, although it can be found in the oral cavity or vaginal tract. [7] It can be resistant to antibiotics (drugs that kill bacteria). When people are immunocompromised (have a weakened immune system) or have an underlying disease, E. faecalis can become pathogenic (disease-causing). For this reason, E. faecalis is considered an opportunistic pathogen—one that takes advantage of the body when immune defenses are low. E. faecalis can enter the body during surgery, insufficiently cleaned medical devices and equipment, improper hand hygiene, or ingesting contaminated foods and fluids. Of the many species of Enterococcus, E. faecalis and Enterococcus faecium (E. faecium) are pathogenic, with E. faecalis being the most prevalent in infections. [7] Common E. faecalis infections include urinary tract infections (UTIs), wound infections, intra-abdominal and pelvic infections, bacteremia (infection in the blood), and endocarditis (inflammation of the heart). If these infections become systemic (widespread), they can cause serious to life-threatening symptoms. [7]

B.2 . Streptococcus viridans:

We have mentioned earlier that the Streptococcus viridans are often present in the mouth, genital regions, and the gut. The most serious infection of Streptococcus viridans occur when the microbe or the bacteria enters any other regions of your body. For instance, in case the Streptococcus viridans enter into the bloodstream, it can result in endocarditis or an infection of the heart's inner lining. It must be mentioned that those people who have damaged heart valves or who suffer from cardiac abnormalities and a compromised immune system, are at a high risk of getting infected by Streptococcus viridans. [8]

C. . Effect of antibiotics:

C.1. Enterococcus faecalis:

It has been observed that various microorganisms are acquiring resistance to most of the available potent antibiotics; hence, there is a need for every hospital to follow the use of antibiotics according to antibiotic sensitivity pattern in that particular hospital or geographical area. It has been reported that Enterobacteriaceae group of microorganisms are increasingly acquiring resistance to many antibiotics and this resistance varies geographically. Enterobacteriaceae were very less sensitive to amoxicillin + clavulanic acid (13.7%), chloramphenicol (7.6%), cefoperazone (14.4%), cefixime (15.7%), and cefuroxime (17.6). Sensitivity to aztreonam was 32.7%.[9]

D. Streptococcus viridans:

Vancomycin, rifampicin, fluoroquinolones and dalfopristin/quinupristin were active against all tested isolates. High level resistance to gentamicin was not seen. Intermediate and high-level penicillin resistance was present in 28.5 and 14.5% isolates, respectively, with 41.3% of the latter group, being also resistant to cefotaxime. Resistance rates to other antimicrobials were as follows erythromycin 38.5%, clarithromycin 33.5%, clindamycin 7.5% and tetracycline 23%.[10]

E. Aim of study:

The current study was performed to determine the incidence of antibiotic resistance and virulence determinants in Enterococcus faecalis and Streptococcus viridans sourced from clinical samples isolates against various types of commonly used antibiotics in Al-Jalaa Hospital for surgery and accidents from January to March 2022. And its defined as the imbalance between intake and requirement which results in altered metabolism , impaired function and loss of body mass [(2)] or as a state of nutrition in which a deficiency or Imbalance of energy, protein, and different vitamins reasons measurable destructive outcomes on tissue and/ or frame form.[(3)]

II. METHODS AND MATERIALS

A. Study Populations:

The study style was prospective, data-based and enclosed 100 samples of patients in Al-Jalaa Hospital for surgery & accidents –Benghazi from January to March in 2022. When microscopic examination & samples were civilized for microorganism identification and antibiotic susceptibility.

B. Laboratory analysis:

Cultures of samples were performed at the Al-Jalaa Hospital for surgery & accidents Laboratories in Benghazi. Once the

samples were collected, they were transported in trans-isolate medium at temperature to the Al-Jalaa Hospital Laboratories. Sediment from a centrifuged specimen of blood was cultivated on nutrient agar (BA), MackConkey agar (MA) and vitox-enriched chocolate agar (CA) plates. Plates were incubated for 24–48h at 35 °C in an aerobic atmosphere (BA *and MA), an* anaerobic atmosphere (BA) or in an apparatus at a gas concentration of 5% greenhouse emission (CA). Isolates from cultures were identified by normal methods. All microorganism isolates were tested for in vitro antibiotic disc sensitivity.

C. Statistical analysis:

The data were analyzed by SPSS 22.

D. . Treatment:

Antibiotic treatment varied from patient to patient. The antibiotics were used throughout the study amount for all specimens therefore AMC- amoxicillin, CXM-cefuroxime, LEV-levofloxacin, E-Erythromycin, GN- Gentamycin, NA-Nalidixic acid, CRO- Rocephin, AM- Ampicillin, AZM-Azithromycin, TE-tetracycline, and CIP-Ciprofloxacin, were the antibiotics that incontestable in-vitro activity often against all culture organisms.

III. RESULTS AND DISCUSSION

A. Results of socio-demographic data:

This cross-sectional study was on a group of 100 of children and adults in the age group from 14 days to over 46 years in laboratory department of Al-Jalaa hospital for surgery and accidents from 60 urine samples, 3 stool samples and 27 swab samples and 10 semen samples.

A.1 Age group:

The study subjects were between 1 day and over 46 years divided into 4 age groups, the first group (0-14) 8%, the second group (15-30 years) 15%, the third group (30-45 years) 68% and the fourth group (+46 years) 9% as shown in the table (I) & figure (I).

TABLE I. *Distribution of age included in the study*

Age	Percent	Frequency
0-14	8.0	8
15-30	15.0	15
30-45	68.0	68

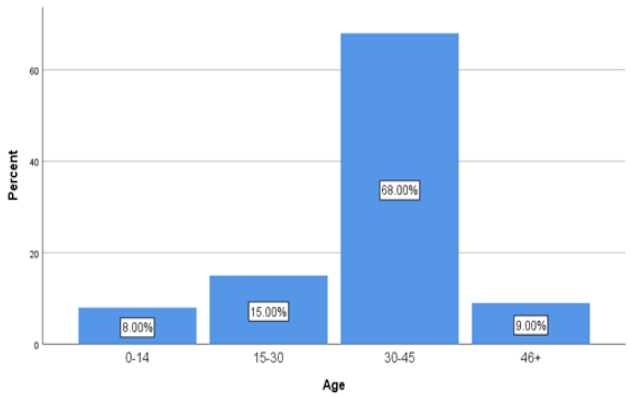

Figure I: *Distribution of age included in the study*

A.1 Sex:

Among 100 investigated, different world about 31 (31.0%) were males and 69 (69%) were females as shown in the table (II) & figure (II).

TABLE II. *Distribution of sex included in the study**:***

Sex		
Percent	Frequency	Samples
31.0	31	Male
69.0	69	Female

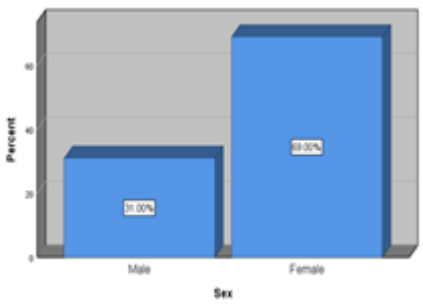

Figure II: *Distribution of sex included in the study*

B. The relation between the age and sex:

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As results and in the table investigated sex and age as shown in the tables (III&IV) & figure (III). The following table shows the significant relationship between age and sex, where the significant value was 0.01, which is less than 0.05.

TABLE (III): *The relation between the age and sex*

The Cross tabulation of age & sex		
Age	**Sex**	
	Female	**Male**
0-14	8	0
15-30	8	7
30-45	50	18
Over 46	3	6
Total	69	31

TABLE (IV): *The relation between age & sex included in the study by chi-square tests:*

Chi-Square Tests			
Asymptotic Significance (2sided)		***Df***	***Value***
0.010	***3***	***11.320[a]***	***Pearson Chi-Square***
a. 3 cells (37.5%) have expected count less than 5. The minimum expected count is 2.48.			

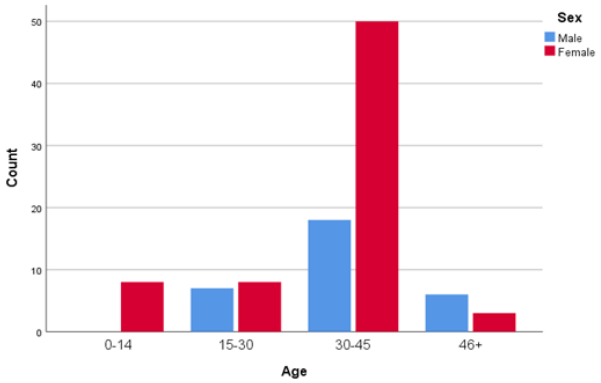

FIGURE (III): *The relation between age & sex included in the study.*

C. Culture results:

Microscopic examination for samples, gram staining and culture for bacteria for the 100 sample that was positive to culture test, found that of 100 samples causes positive infection urine 55(55%) stool 3(3%) swab 27(27%) urine RIE 5 (5%) semen 10 (10%) as shown in the table (V) & figure (IV).

TABLE V. *Microscopy and culture results.*

Samples	Percent	Frequency
Urine	55.0	55
Stool	3.0	3
Swab	27.0	27
Urine RIE	5.0	5
Semen	10.0	10
Total	100%	100

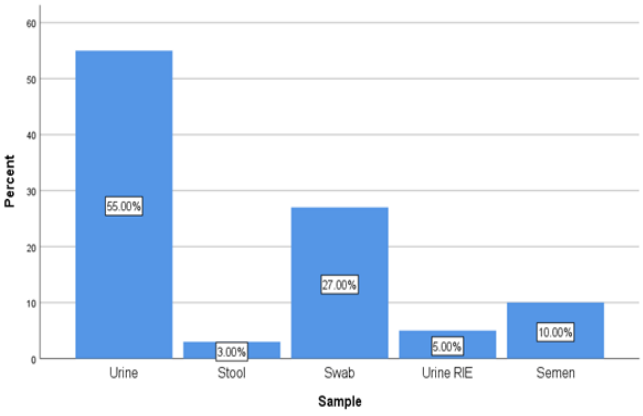

FIGURE. (IV): *Microscopy and culture results*

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D. Culture results of bacterial isolates:

As results and in the table investigated sex and samples isolated as shown in the tables (VI&VII) & figure (V). The following tables show the significant relationship between age and sex, where the significant value was 0.00, which is less than 0.05. The isolated samples are 50 samples for *Enterococcus faecalis* & 50 samples for *Streptococcus viridans*

TABLE (VI): *The relation between sex & samples included in the study*.

The cross tabulation of sex * sample						
Sex	Sample					**Total**
	Semen	**Urine RIE**	**Swab**	**Stool**	**Urine**	
Male	10	0	15	3	3	31
Female	0	5	12	0	52	69
Total	10	5	27	3	55	100

TABLE (VII): *The relation between sex & samples by Chi-square tests***:**

Chi-Square Tests			
Asymptotic Significance (2-sided)	**df**	**Value**	
0.000	4	55.573[a]	Pearson Chi-Square
a. 5 cells (50.0%) have expected count less than 5. The minimum expected count is .93.			

ISSN: 2789-2220

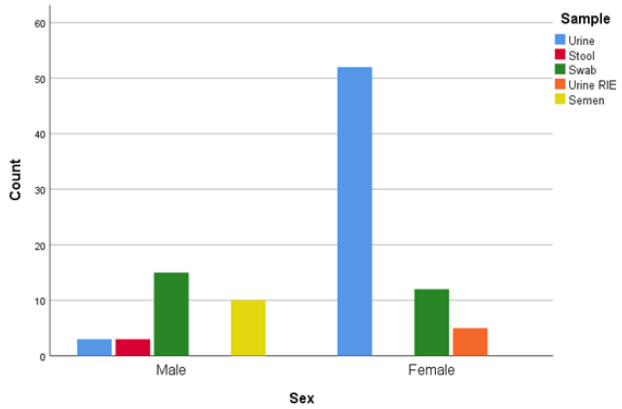

FIGURE (5): *The relation between sex & samples included in the study.*

E. Enterococcus faecalis :

As show in the figure (VI) these from we shown that the *Enterococcus faecalis* is gram positive, In the figure (VII) this hemolysis alpha in Macconkey agar show the bacteria is *Enterococcus faecalis* gram positive. From the table (VIII) & the figure (VIII), we show that the more sensitive antibiotics for the *Enterococcus faecalis* is LEV-levofloxacin and more resistant antibiotics is AMC-amoxicillin

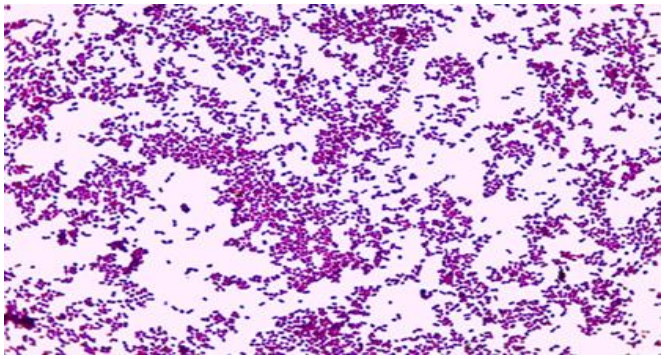

FIGURE (VI): *The gram stain of Enterococcus faecalis*

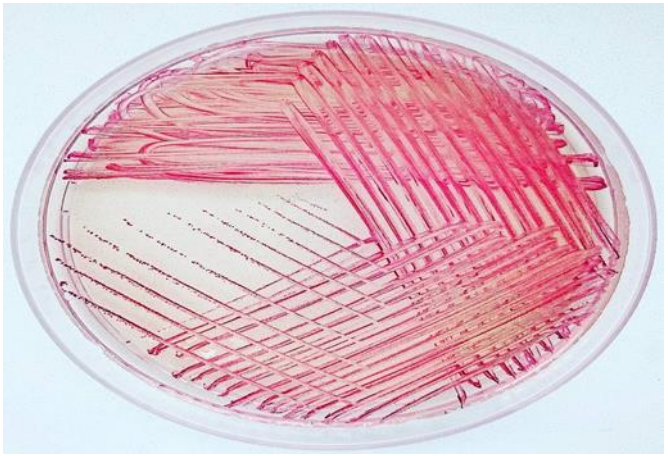

FIGURE (VII): *The growth of Enterococcus faecalis on Macconkey agar*

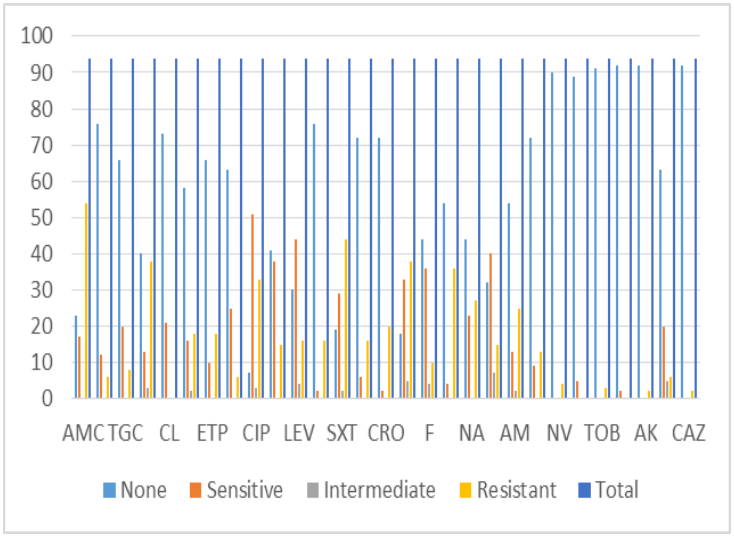

FIGURE (VIII): *The antibiotics sensitivity test for Enterococcus faecalis*

ISSN: 2789-2220

TABLE (VIII): *The antibiotic sensitivity test for Enterococcus faecalis*

Antibiotic	None	Sensitive	Intermediate	Resistant	Total
AMC	23	17	0	54	94
IPM	76	12	0	6	94
TGC	66	20	0	8	94
CXM	40	13	3	38	94
CL	73	21	0	0	94
FEP	58	16	2	18	94
ETP	66	10	0	18	94
C	63	25	0	6	94
CIP	7	51	3	33	94
TE	41	38	0	15	94
LEV	30	44	4	16	94
CIV	76	2	0	16	94
SXT	19	29	2	44	94
CTX	72	6	0	16	94
CRO	72	2	0	20	94
AZM	18	33	5	38	94
FA	44	36	4	10	94
AX	54	4	0	36	94
NA	44	23	0	27	94
GN	32	40	7	15	94
AM	54	13	2	25	94
CFM	72	9	0	13	94
NV	90	0	0	4	94
CZ	89	5	0	0	94
TOB	91	0	0	3	94
PRL	92	2	0	0	94
AK	92	0	0	2	94
D0	63	20	5	6	94
CAZ	92	0	0	2	94

F. Streptococcus viridans:

As show in the figure (IX) these from we shown that the Viridans Streptococci is gram positive, In the figure (X) this hemolysis alpha in Macconkey agar show the bacteria is Viridans Streptococci gram positive, From the table (VIII) & the figure (XI) we show that the more sensitive antibiotics for the Viridans Streptococci is CIP-ciprofloxacin and LEV-levofloxacin, and more resistant antibiotics is AMC-amoxicillin.

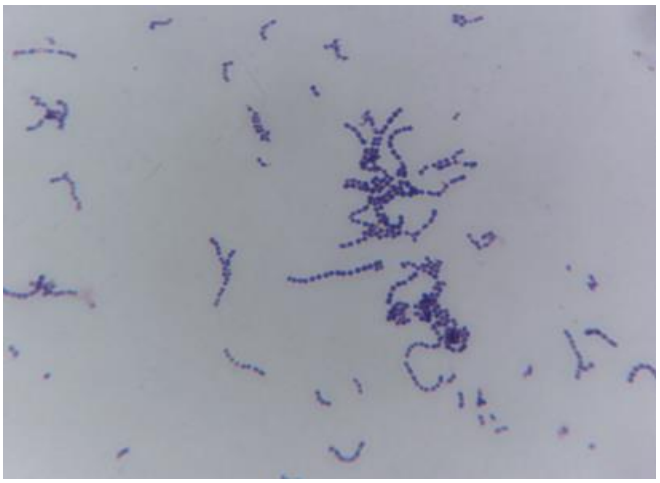

FIGURE (IX): *The gram stain of Streptococcus viridans*

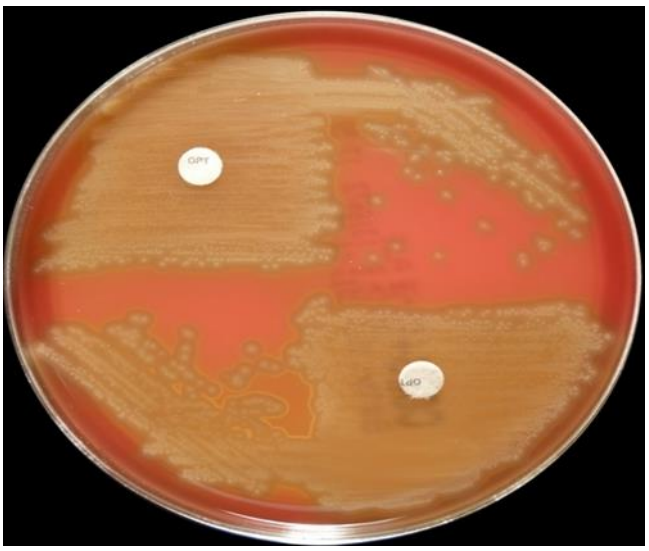

FIGURE (X): *The growth of Streptococcus viridans on Macconkey agar*

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TABLE (IX): *The antibiotics sensitivity test for Streptococcus viridans:*

Antibiotic	None	Sensitive	Intermediate	Resistant
AMC	22	19	0	59
IPM	85	7	0	8
TGC	73	27	0	0
CXM	33	20	2	45
CL	79	21	0	0
FEP	80	4	0	16
ETP	84	3	0	13
C	83	17	0	0
CIP	5	58	6	31
TE	32	48	0	20
LEV	21	57	5	17
CIV	75	10	0	15
SXT	35	21	3	41
CTX	74	4	0	22
CRO	60	7	0	33
AZM	30	29	5	36
FA	48	37	5	10
AX	58	0	0	42
NA	31	44	0	25
GN	35	40	8	17
AM	62	11	0	27
CFM	85	4	0	11
CZ	86	8	0	6
NV	88	5	2	5
DO	67	21	3	9

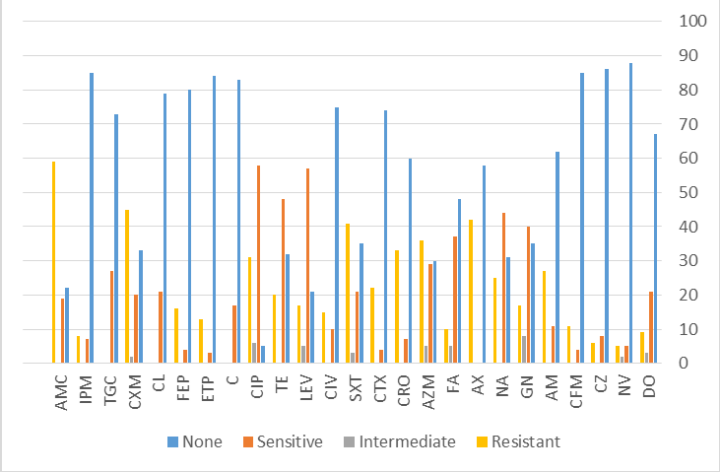

FIGURE (XI) :*The antibiotics sensitivity test for Streptococcus viridans.*

Antibiotic resistance is a global health threat and believed to contribute to 35,000 deaths every year in the United States. In this study, among 31 samples from males and 69 samples from women suspected of having gram-positive bacteria in Al-Jalaa Hospital for surgery & accidents (Benghazi), 31.0% of the male samples and 69.0% of the female samples were positive.
In our study Viridans Streptococci has showed a high resistance to AMC-amoxicillin and CXM-cefuroxime which is not similar to the study reported by Nidhi Singh, Linda Progeneses 2022 [9]. And in our study *Enterococcus faecalis* has shown resistance high to AMC-amoxicillin and SXT-Sulfamethoxazole which is in similar with the study reported by I.U. Rathnayake (2012) [8].

There are many strengths of this project including the large number of isolations, the wide range of antibiotics included, and the data disaggregated by age and gender. These differences were drastic for a few antibiotics and warrant a future study to determine whether one species is more resistant than the others. Thus, some of the changes in susceptibility over time may not apply to all isolates from all culture sites or types of infection.

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IV. CONCLUSION

In conclusion, we observed susceptibility rates of many clinically relevant antibiotics for Viridans Streptococci and *Enterococcus faecalis* to commonly used antibiotics.

This difference in susceptibility suggests that it may be beneficial to routinely define the species of Viridans Streptococci and *Enterococcus faecalis* isolates in the clinical microbiology laboratory to better facilitate antibiotic selection. Of great concern, there was a significant trend toward decreased susceptibility to AMC-amoxicillin and CXM-cefuroxime for Viridans Streptococci isolates and AMC-amoxicillin and SXT-Sulfamethoxazole for *Enterococcus faecalis* isolates. There was also a trend toward decreased penicillin susceptibility among Viridans Streptococci and *Enterococcus faecalis* isolates from all culture sites but not among the subset of isolates from the blood.

Continuation of these trends could have important implications in the treatment of Viridans Streptococci and *Enterococcus faecalis* infections and warrants continued monitoring. Empirical antibiotic selection must consider potential changes in Viridans Streptococci and *Enterococcus faecalis* susceptibility patterns to maintain adequate clinical response. [12]

REFERENCES

[1] Almeida, Carolina Vieira; Taddei, Antonio; Amedei, Amedeo (2018-01-01). "The controversial role of Enterococcus faecalis in colorectal cancer". Therapeutic Advances in Gastroenterology. SAGE Publications. 11:175628481878360. doi:10.1177/1756284818783606. ISSN 1756-2848. PMC 6044108. PMID 30013618.

[2] Ryan KJ, Ray CG, eds. (2004). Sherris Medical Microbiology (4th ed.). McGraw Hill. pp. 294–295. ISBN 0-8385-8529-9.

[3] Molander, A.; Reit, C.; Dahlén, G.; Kvist, T. (1998). "Microbiological docks of root-filled teeth with apical periodontitis". International Endodontic Journal. 31 (1):1–7.

[4] Rocas, I.; Siqueira, J.; Santos, K. (2004). "Association of Enterococcus faecalis With Different Forms of Periradicular Diseases". Journal of Endodontics. 30 (5):315–320.

[5] Sinner, Scott W.; Tunkel, Allan R. Bennett, John E.; Dolin, Raphael; Blaser, Martin J. (eds.), (2015-01-01). "204 - Viridans Streptococci, Nutritionally Variant Streptococci, Groups C and G Streptococci, and Other Related Organisms", Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases (Eighth Edition), Philadelphia: W.B. Saunders, pp. 2349–2361

[6] Dorland's Illustrated Medical Dictionary, (2019). Headword "streptococcus", subentry "viridans streptococci".

[7] Anderson AC, Jonas D, Huber I, et al. (2016). "Enterococcus faecalis from food, clinical specimens, and oral sites: prevalence of virulence factors in association with biofilm formation. " Frontiers in Microbiology. 2016;0. doi:10.3389/fmicb.2015.01534.

[8] Kerkar PD, Fligiel S, McMath L, Kundu S, Motamidi M, Spears, R, Haas GP (1990) "Laser balloon dilatation of the ureter: Histological and electron microscopical study of normal tissue effects". Presented at American Urological Association on Oct.

[9] Summaiya Mulla, Jaykaran Charan1, Tanvi Panvala (2011) "Departments of Microbiology and Pharmacology", Govt. Medical College, Surat, Gujarat, India.

[10] Sofia Ioannidoua P. T. Tassiosb A. Kotsovili, Tseleni. Foustoukoua N. J. Legakisb A. Vatopoulosc (2001)."Antimicrob Agents". 2001 Mar;17(3):195-201.