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# ISOLATION AND ANALYSIS OF AMOXICILLIN RESISTANT BACTERIA FROM DRINKING WATER SAMPLES

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## ABSTRACT

Antibiotics are necessary evils. They are desired entities and sustainability of life is not possible without the use of antibiotics. Humans had been using antibiotics for a long. As a result, resistance had been reported against certain effective antibiotics. Keeping in view the importance of resistance development against antibiotics, the present studies were planned to identify microbial population in drinking water against broad-spectrum antibiotic amoxicillin of different brands GSK (Glaxo SmithKline) and CSPC (Shijiazhuang Pharma Group). Water samples were collected from different areas of Faisalabad, Pakistan. Every hundred microliters of samples were spread out on LB media plates, augmented with specific concentrations of antibiotics. In the present study, two different brands of amoxicillin (GSK and CSPC) were used and the microbial population was compared on LB plates having an appropriate concentration of antibiotic. The bacterial plates were grown on different concentrations of amoxicillin and the highest concentration was used in this study was 36µg/mL. The highly resistant bacteria strains were investigated by biochemical and molecular analysis. Different species were identified like streptococcus, staphylococcus, streptobacillus, etc. All the identified isolates showed higher resistance against CSPC and GSK. The potential of the examined water samples and their spreading effect on public health was discussed. The highest concentration of pollutants was identified in Gurunanakpura, Guttwala, UAF, and Sir Syed town as compared to other samples. The highly resistant bacterial strains involving in the prognosis of harmful diseases making the water unfit for human consumption

**Keywords:** Amoxicillin**,** Streptococcus, Staphylococcus, Streptobacillus, Pollutants, Antibiotic Resistance

## INTRODUCTION:

In many countries, people use antibiotics in an unrestricted way and without the prescription of doctors because self-medication is habitual. So due to the overuse and misuse, many bacterial communities are highly influenced by the expulsion of antibiotics in the aquatic environment leads to various sort of antibiotic resistances (1,2). Antibiotic resistance genes (ARGs) detected as a consequence of resistance to several antibiotics in water environment (3). Many antibiotics used for the remedy of infection emerge from the environment; induce by microorganisms. The aquatic environment is the main source of antibiotic resistance, plays a role in the proliferation of antibiotic resistance genes (5, 6). In recent studies, it is emphasized that the aquatic environment is adversely affected by the contamination of antibiotics responsible for environmental pollution, because it affects directly human health and the environment (7,8).

Contaminated drinking water can exhibit people to a variety of pathogenic pollutants. Microorganisms like bacteria, parasites, viruses contaminate the drinking water which leads the public to various harmful diseases like diarrhea, gastrointestinal infection, nausea, cramps, typhoid, etc. Many kinds of nitrate, fluoride, heavy metals, organic chemicals are also contaminating the drinking water which exposes a very serious syndrome-like cancer (EPA, USA).

According to the world health organization (9,10) almost 840,000 people died each year due to diarrhea as a consequence of contaminated drinking water. About 240 million people are affected because some kinds of parasitic worms are also present in contaminated drinking water which causes many chronic and acute diseases like ‘schistosomiasis. Some kinds of insects that live in water responsible for dengue fever. Sometimes our body does not completely metabolize the antibiotics, so this antibiotic discharges into the environment in authentic form (11). This is the main concern of the existing and increasing concentration of antibiotics in the environment. Due to the presence of antibiotics, it automatically possesses antibiotic resistance genes into the environment. It helps bacteria and other pathogens to easily get resistant against these antibiotics (12, 13). The antibiotic resistance genes are harmful to both environment and humans because they are transferred genetically in bacteria and pathogens (14). Recent studies emphasize the main key points of the prevalence of antibiotics in the environment, one is the improper metabolization and the other is the irregular junk of antibiotics in sewage treatment plants (15,16).

The dominant issue arising in Pakistan that people using antibiotics of different brands to treat viral infections like cough, cold, flu, sore throat, and ear infections, because self-medication is very common. So due to the overuse of antibiotics, many sensitive bacteria are killed but resistant bacteria are growing and developed resistance to various antibiotics, able to produce infectious microbial strains. So having all these points plant drinking water having microbial population exists. The objective of this study was designed to isolate the resistant bacteria against amoxicillin of different brands from drinking water samples collected from public places of tap water. To make a comparison of both brands to get an estimation of which brand is more sensitive to bacterial survival.

## MATERIAL AND METHODS

**Study area for sample collection:**

All the drinking water samples were collected from various locations of Faisalabad Punjab Pakistan like ghulam muhammad abad, jhal, University of Agriculture, people's colony, guttwala, gurunanak pura, PIA office and sir syed town. Samples were collected in small plastic bottles from tap water of public places, with a volume of up to 1L in February, March, and April. For further analysis, all the samples were placed at 4°C. Amoxicillin of two different brands GSK (GlaxoSmithKline) 500mg and CSPC 500mg (Shijiazhuang Pharma Group) were selected. Antibiotics of both brands were filtered by using the syringe filtration method before use.

**Experimental analysis of samples:**

Experimental analysis was done by using different water parameters like EC (µs/cm), pH, TDS (Total Dissolved Solids) (mg/L), Chlorides (mg/L), Coliform (cfu/100mL), *E. coli* (cfu/100mL) showing in Table 1. All the values were compared with values given by WHO (World Health Organization). The purpose of the analysis was to ensure the quality of water that people use for drinking purposes.

**Bacteriological Analysis:**

The bacteriological study was performed by using 400mL lysogeny broth media enriched with the concentration of amoxicillin 4µg/mL by adding 8µL GSK in 200mL L.B media and 4µL CSPC in another 200mL L.B media by using formula MIVI and M2V2. After pouring in 16 Petri plates different drinking water samples of 100mL were spread out by using the spreader and incubated at 32°C. After 16 hours colonies were counted by using colony counter. Same procedure was applied for 8µg/mL (16µL GSK, 8µL CSPC), 16µg/mL (32µL GSK, 16µL CSPC), 32µg/mL (64µL GSK, 32µL CSPC), 36µg/mL (72µL GSK, 36µL CSPC). After counting the total no. of colonies, steaking was performed to isolate pure bacterial strains from single species of microbes. Then culturing of bacterial isolates was performed by using liquid culture media by picking a single colony with the help of a toothpick and dropped in a tube having 3mL liquid culture media. In the case of culturing e.g., if a colony was picked from 4µg/mL concentrated plates then they dropped in the tube with the same concentration of liquid media in a tube. Total no. of 30 cultures were incubated at 30°C in a shaking incubator for

16 hours. All the cultures were preserved by adding glycerol solution at -80°C.

**Biochemical study and structural observation of bacteria.**

Biochemical analysis was performed by testing a different culture to make differentiate between anaerobic and aerobic bacteria. Because catalase enzyme present in aerobic cells involved in the breakdown of $H_2O_2$ in water and oxygen. A small quantity of culture was added on a glass slide and 3% hydrogen peroxide was mixed with the culture. The formation of bubbling showed positive results. Total no. of 30 cultures were studied under the catalase test in which 12 cultures showed positive results and the remaining 18 cultures were catalase-negative. For identification of resistant bacterial survival against amoxicillin, gram staining was performed to observe the strains and their structures.

**DNA extraction of different bacterial isolates:**

The DNA extraction was performed by the phenol-chloroform method[16]. For isolation, a culture of 3mL was taken in an Eppendorf tube and spun at 13300rpm. Then ice cold T.E buffer was added with the concentration of 567μL and 10% SDS of 30μL for lysis. The culture was incubated at 37°C for 2 hours after the addition of proteinase K for digestion of protein. The additional protein that bound to the DNA was removed by 5M NaCl solution. The CTAB-NaCl solution was incorporated with a concentration of 80μL to separate aqueous from the organic layer. To reduce foaming isoamyl alcohol was centrifuged at 13300rpm. After the transformation of the upper layer, purification was done by phenol-chloroform-isoamyl (25:24:1) 780μL and sodium acetate 3M of 20μL solution. The next step was carried out by the addition of chilled absolute ethanol of 1mL solution for precipitation purposes. After spinning at 13300rpm supernatant was discarded to get DNA pellet. To wash the pellet 250μL of 70% ethanol was used. After discarded supernatant pellet was air-dried and resuspended by adding 30μL $D_3H_2O$.

**PCR amplification of bacterial isolates:**

For PCR amplification oligonucleotide 16S rRNA primers like D1S2816-f with the sequence of 5′ to 3′ (TTTCCCAAATGTATTACTGCATT) D1S2816-r 3′ to 5′ (GAGTACCCAATCCCAGTGC) 909F 5′ to 3′ (AAACTCAAAGGAATTGAC) 909R 3′ to 5′ (GACGGGCGGTGTGTACCAA) were used. Reaction mixture of 0.1 ng DNA in 25μL PCR buffer containing deoxynucleotide triphosphate (dNTPs) 2mM, Taq polymerase 0.1 U, 2mM $MgCl_2$, 1X buffer,0.5 μmol/L of forward and reverse primers. Further amplification is followed by denaturation for 1 min at 95℃, annealing for 1 min at 59℃, and elongation for 2 min at 72℃. The next process is carried out by running PCR products by 1.5% agarose gel in 1X Tris-borate-EDTA.

**Data Analysis:** All results were expressed and analyzed statistically using one-way analysis of variance ANOVA.

## RESULTS

**Experimental analysis of drinking water samples by using different parameters:**

Table 1. showing the different parameters like pH, TDS, chlorides, coliform, etc. The standard pH of drinking water is 6.0-8.5 so a toxic level of pH was not found in any sample. According to WHO the standard concentration of TDS is 250mg/L while chlorides having 1000mg/L. So, the concentration of TDS and chlorides is quite high in various samples. The standard concentration of coliform is 0 cfu/100mL but in water samples, a high amount of concentration was identified in different samples but not observed near PIA and jhal. E. coli was detected in few samples but other forms of drug-resistant bacteria were also distinguished.

**Correlation matrixes of experimental parameters of water:**

Table 2 showing the correlation matrix among different parameters and highly significant 0.966** results were investigated among TDS and chlorides. In Table 3 GSK-T1, TDS showed a highly significant ($P<0.01$) outcome as compared to chlorides and electrical conductivity ($P<0.05$). In the correlation of CSPC, highly significant in terms of pH and significant ($P<0.05$) in TDS and EC was observed (Table 4)

**Bacteriological analysis against amoxicillin of GSK and CSPC:**

Bacterial communities showed a high level of resistance in guttwala sample (Table 5). Also, a significantly highest number of colonies were observed in GM Abad sample (Table 6). Overall, the treatment T4 showing the least number of colonies with the concentration of antibiotic 32 µg/mL (Table 5, 6)

**Biochemical analysis of bacteria**

Table 7 reveals the percentage of presence of different bacteria in the sample. The percentage of total gram-positive staphylococcus was high (63.30%) as compared to the gram-negative isolate. In the catalase test, the percentage of catalase -ve is high (53.30%) as compared to catalase +ve (46.70%) (Table 8). Overall, 46.70% gram-positive bacteria were catalase-positive and 53.30% were catalase-negative in the relationship between catalase test and gram staining (Table 9)

**Comparison of average no. of bacterial colonies against the different concentrations of amoxicillin brand GSK and CSPC**

A among all the GSK treatments guttwala showed significantly highest no. of colonies as compared to the others (Fig. 1A). In the case of CSPC significantly high no. of colonies were identified in GM Abad (Fig. 1B). Overall bacterial strains are more resistant to CSPC and GSK with increasing the concentration of antibiotics (Fig. 1C)

**Molecular analysis of resistant bacteria:**

DNA isolation of different bacterial strains was performed like Sa: Streptococcus, Sb: Streptobacillus, and Sc: Staphylococcus (Fig. 1A). PCR amplified of 130 bp amoxicillin-resistant bacterial strains were identified (Fig. 1B).

## Discussion

Different water samples were collected to identify bacterial resistance against antibiotics of different brands of amoxicillin. All drinking water samples showed pH under the certain limit of WHO which was 6.0-8.5. The value of pH below 6.0 ceases the activity of various vitamins in the human body and pH above 8.5 causes skin diseases (17,18). Among all the samples, the highest electrical conductivity was identified in UAF which was 1384µs/cm. Water showing higher electrical conductivity if there are more ions present in water, the lesser the ion the less conductivity of water (19). According to the WHO, the standard concentration of TDS (total dissolved solids) must be 250 mg/L but in this study the highest values of TDS identified in the case of sir syed town which was 3520 mg/L, and in gurunanakpura which was 3545. Any alteration in the activity of inorganic and organic material (TDS) causes many notorious effects on the human body (20). The highest range of chlorides is identified in the sample of gurunanakpura which was 1270 that is exceeding the standard limit of WHO. The highest chlorides concentration indicating that the water is polluted badly (21). The value of coliform and E.coli in drinking water must be 0 cfu/100mL (WHO). The highest exceeding limit of the coliform was identified in gutwala (1600/100mL) and gurunanakpura (1800/100mL) but all drinking water samples are free from E. coli. Presence of coliform indicating the contact of drinking water with sewage system(22).

All the isolated bacteria (Staphylococcus, Streptococcus, Streptobacillus) showed high resistance against broad-spectrum antibiotic amoxicillin. The results are contradicting with the study of Adesoji et al (23) who reported the least resistance of staphylococcus in water and concluded that the least resistance might be due to the pre-treatment process with antiseptics. Staphylococcus is imposing serious threats to the public which normally resides in drinking water. Its infectious strains are strongly involved in antibiotic resistance (24,25) Resistance of streptococcus also similar to various studies in which the resistance of Streptococcus has been seen against antibiotics like erythromycin, penicillin, and amoxicillin(26). Resistance has reported (27) in bacteria through many possible ways like agricultural wastes, irregular junk of antibiotics in water, sewage system, excrements of humans and animals, etc. Resistance of streptobacillus has also been reported (28) against cadmium when isolated from drainage water.

In developing countries, proper checks and balances should introduce to minimize the usage of least effective antibiotics. To reduce morbidity and mortality different conditions should be improved in water distribution systems like sanitary, disposal of pharmaceutical slaughter and animal wastes, etc. This study will further help the researcher to uncover the mechanism and approaches to reduce bacterial resistance against amoxicillin. Thus, a new theory on bacterial resistance may be arrived at.

## CONCLUSION

All the bacterial isolates streptococcus, staphylococcus, streptobacillus showed the highest resistance against both brands of antibiotic (GSK, CSPC). The identified bacterial strains are involved in the prevalence of many harmful diseases like abscesses, bloodstream infections, pharyngitis, cellulitis, arthritis, rash, etc. While in some treatments bacterial strains showed less resistance against GSK as compared to CSPC. The highest concentration of TDS, chlorides, and coliform has been also seen in some samples like gurunanakpura, sir syed town, gut wala, etc. This study showed that the drinking water resources are polluted and involved in the prognosis of many harmful diseases which make the water unfit for human consumption.

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**Table 1: Experimental analysis of different water parameters**

| **Parameters** | **Near PIA** | **Gut Wala** | **Ghulam Muhammad Abad** | **Peoples Colony** | **Sir Syed Town** | **Jhal** | **UAF** | **Gurunanak pura** | **WHO Standards for drinking water** |
|---|---|---|---|---|---|---|---|---|---|
| pH | 7.80 | 7.60 | 7.60 | 7.00 | 7.40 | 7.90 | 7.80 | 7.10 | 6.0-8.5 |
| EC (µs/cm) | 891 µs/cm | 259 µs /cm | 830 µs/cm | 293 µs/cm | 5.50 ms/cm | 506 µs/cm | 1384 µs/cm | 5.54 ms/cm | NG |
| TDS (mg/L) | 570 | 165 | 531 | 187 | 3520 | 323 | 885 | 3545 | 250 |
| Chlorides (mg/L) | 60 | 50 | 150 | 100 | 870 | 60 | 130 | 1270 | 1000 |
| *Coliform* (cfu/100mL) | Nil | 1600 | 700 | 72 | 30 | Nil | 1200 | 1800 | 0cfu/100 mL |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *E.coli* (cfu/100mL) | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | 0cfu/100 mL |

TDS: Total dissolved solid EC: electrical conductivity

**Table: 2 Correlation matrixes of water parameters**

| | pH | EC | TDS | Chlorides | Coliform |
|---|---|---|---|---|---|
| **pH** | 1.000 | | | | |
| | | | | | |
| | | | | | |
| **EC** | 0.512 | 1.000 | | | |
| | 0.195 | | | | |
| | | | | | |
| **TDS** | -0.436 | -0.018 | 1.000 | | |
| | 0.280 | 0.965 | | | |
| | | | | | |
| **Chlorides** | -0.562 | -0.132 | 0.966** | 1.000 | |
| | 0.147 | 0.756 | 0.000 | | |
| | | | | | |
| **Coliform** | -0.171 | 0.068 | 0.210 | 0.338 | 1.000 |
| | 0.686 | 0.872 | 0.617 | 0.413 | |

Upper values indicated Pearson's correlation coefficient; Lower values indicated a level of significance at 5% probability.

* = Significant ($P<0.05$); ** = Highly significant ($P<0.01$)

**Table: 3 Correlation among pH, EC, TDS, Chlorides, Coliform with GSK at different treatments and the overall mean.**

| | pH | EC | TDS | Chlorides | Coliform |
|---|---|---|---|---|---|
| **GSK_T1** | -0.350 | -0.065 | 0.863** | 0.796* | 0.006 |
| | 0.395 | 0.878 | 0.006 | 0.018 | 0.988 |
| | | | | | |
| **GSK_T2** | -0.322 | -0.658 | -0.159 | -0.118 | 0.273 |
| | 0.437 | 0.076 | 0.707 | 0.781 | 0.512 |
| | | | | | |
| **GSK_T3** | 0.461 | -0.260 | -0.145 | -0.174 | -0.384 |
| | 0.251 | 0.534 | 0.731 | 0.680 | 0.348 |
| | | | | | |
| **GSK_T4** | -0.366 | -0.556 | -0.321 | -0.311 | -0.240 |
| | 0.373 | 0.152 | 0.439 | 0.453 | 0.567 |
| | | | | | |
| **GSK_mean** | -0.314 | -0.739* | 0.068 | 0.081 | 0.177 |

| | | | | | |
|---|---|---|---|---|---|
| | 0.449 | 0.036 | 0.872 | 0.848 | 0.675 |

Upper values indicated Pearson's correlation coefficient; Lower values indicated a level of significance at 5% probability.

T1, T2, T3, T4: Treatments

* = Significant ($P<0.05$); ** = Highly significant ($P<0.01$)

**Table: 4 Correlation among pH, EC, TDS, Chlorides, Coliform with CSPC at different treatments and the overall mean.**

| | pH | EC | TDS | Chlorides | Coliform |
|---|---|---|---|---|---|
| **CSPC_T1** | 0.050 | -0.516 | 0.300 | 0.245 | 0.118 |
| | 0.906 | 0.190 | 0.470 | 0.559 | 0.780 |
| | | | | | |
| **CSPC_T2** | -0.067 | -0.124 | 0.119 | 0.116 | 0.196 |
| | 0.875 | 0.771 | 0.778 | 0.784 | 0.642 |
| | | | | | |
| **CSPC_T3** | 0.311** | -0.391 | 0.043 | -0.009 | -0.374 |
| | 0.454 | 0.338 | 0.920 | 0.982 | 0.361 |
| | | | | | |
| **CSPC_T4** | 0.076 | -0.297 | 0.257* | 0.113 | -0.093 |
| | 0.858 | 0.476 | 0.540 | 0.790 | 0.826 |
| | | | | | |
| **CSPC_mean** | 0.002 | -0.318* | 0.200 | 0.172 | 0.146 |
| | 0.996 | 0.443 | 0.635 | 0.684 | 0.730 |

Upper values indicated Pearson's correlation coefficient; Lower values indicated a level of significance at 5% probability.

T1, T2, T3, T4: Treatments

* = Significant ($P<0.05$); ** = Highly significant ($P<0.01$)

**Table: 5 Analysis of resistant bacteria against amoxicillin brand GSK at different treatment levels**

| **Samples** | **Treatment** | | | | **Mean** |
|---|---|---|---|---|---|
| | **T1 (4 µg/mL)** | **T2 (8 µg/mL)** | **T3 (16 µg/mL)** | **T4 (32 µg/mL)** | |
| **G.M Abad** | 160 | 280 | 2 | 0 | 110.50±67.80 |
| **Jhal** | 19 | 2 | 258 | 0 | 69.75±62.89 |
| **UAF** | 17 | 0 | 3 | 0 | 5.00±04.06 |
| **Peoples colony** | 15 | 500 | 2 | 5 | 130.50±123.2 |
| **Guttwala** | 25 | 1022 | 43 | 4 | 273.50±249.6 |
| **Gurunanak pura** | 200 | 120 | 6 | 0 | 81.50±48.19 |

| | | | | | |
|---|---|---|---|---|---|
| **PIA** | 60 | 8 | 16 | 3 | 21.75±13.03 |
| **Sir Syed Town** | 302 | 408 | 64 | 2 | 194.00±96.27 |
| **Mean** | 99.75 | 292.50 | 49.25 | 1.75 | |
| | ±38.34 | ±124.60 | ±30.88 | ±0.73 | |

Means sharing similar letters in a row or a column are statistically non-significant (P>0.05).

**Table: 6 Analysis of resistant bacteria against amoxicillin brand CSPC at different treatment levels**

| **Sample** | **Treatment** | | | | **Mean** |
|---|---|---|---|---|---|
| | **T1 (4 µg/mL)** | **T2 (8 µg/mL)** | **T3 (16 µg/mL)** | **T4 (32 µg/mL)** | |
| **G.M Abad** | 170 | 1000 | 3 | 0 | 293.25±238.9 |
| **Jhal** | 250 | 5 | 156 | 0 | 102.75±60.99 |
| **UAF** | 28 | 2 | 1 | 0 | 7.75±06.76 |
| **Peoples colony** | 25 | 31 | 13 | 0 | 17.25±06.86 |
| **Guttwala** | 400 | 500 | 45 | 5 | 237.50±124.6 |
| **Gurunanakpura** | 150 | 220 | 16 | 0 | 96.50±53.16 |
| **PIA** | 10 | 9 | 4 | 3 | 6.50±01.76 |
| **Sir Syed Town** | 410 | 511 | 83 | 6 | 252.50±122.8 |
| **Mean** | 180.38 | 284.75 | 40.13 | 1.75 | |
| | 57.20 | 127.61 | 19.28 | 0.90 | |

Means sharing similar letters in a row or a column are statistically non-significant (P>0.05).

**Table 7: Relationship between gram staining and identified samples resistant against amoxicillin**

| **Identified samples** | | **Gram Staining** | | **Total** |
|---|---|---|---|---|
| | | **-ve** | **+ve** | |
| **Staphylococcus** | Count | 0 | 10 | 10 |
| | % within Identification of samples | 0.00% | 100.00% | 100.00% |
| | % within Gram Staining | 0.00% | 52.60% | 33.30% |
| **Streptobacillus** | Count | 11 | 0 | 11 |
| | % within Identification of samples | 100.00% | 0.00% | 100.00% |
| | % within Gram Staining | 100.00% | 0.00% | 36.70% |
| **Streptococcus** | Count | 0 | 9 | 9 |
| | % within Identification of samples | 0.00% | 100.00% | 100.00% |
| | % within Gram Staining | 0.00% | 47.40% | 30.00% |
| **Total** | Count | 11 | 19 | 30 |
| | % within Identification of samples | 36.70% | 63.30% | 100.00% |
| | % within Gram Staining | 100.00% | 100.00% | 100.00% |

Chi-square value = 30.00**

P-value = 0.000
** = Highly significant (P<0.05)

**Table: 8 Relationship between Catalase test and Identified bacteria resistant to amoxicillin**

| Identified samples | | Catalase Test | | Total |
|---|---|---|---|---|
| | | -ve | +ve | |
| **Staphylococcus** | Count | 4 | 6 | 10 |
| | % within Identification of samples | 40.00% | 60.00% | 100.00% |
| | % within Gram Staining | 25.00% | 42.90% | 33.30% |
| **Streptobacillus** | Count | 7 | 4 | 11 |
| | % within Identification of samples | 63.60% | 36.40% | 100.00% |
| | % within Gram Staining | 43.80% | 28.60% | 36.70% |
| **Streptococcus** | Count | 5 | 4 | 9 |
| | % within Identification of samples | 55.60% | 44.40% | 100.00% |
| | % within Gram Staining | 31.30% | 28.60% | 30.00% |
| **Total** | Count | 16 | 14 | 30 |
| | % within Identification of samples | 53.30% | 46.70% | 100.00% |
| | % within Gram Staining | 100.00% | 100.00% | 100.00% |

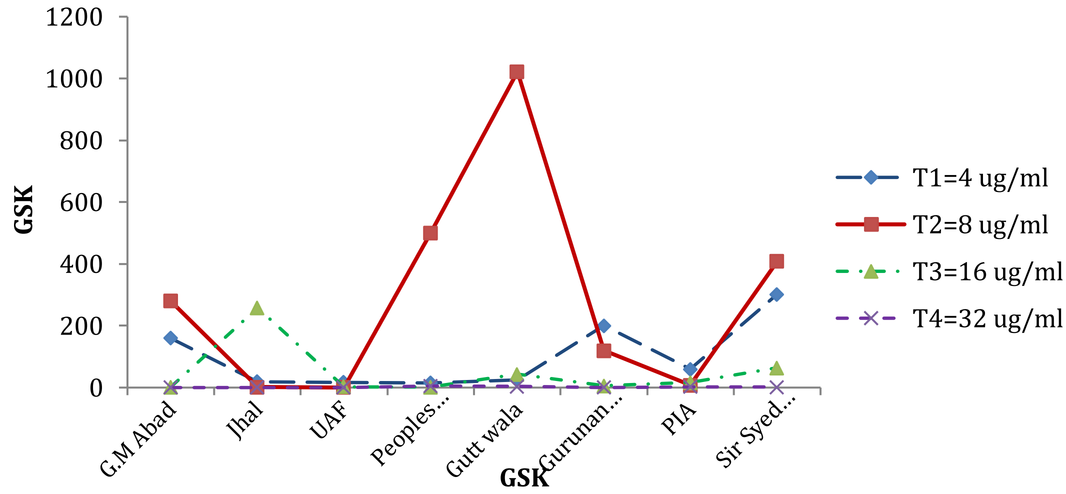
Chi-square value = $1.201^{NS}$
P-value = 0.548
NS = Non-significant (P>0.05)

**Table 9: Relationship between catalase test and gram staining.**

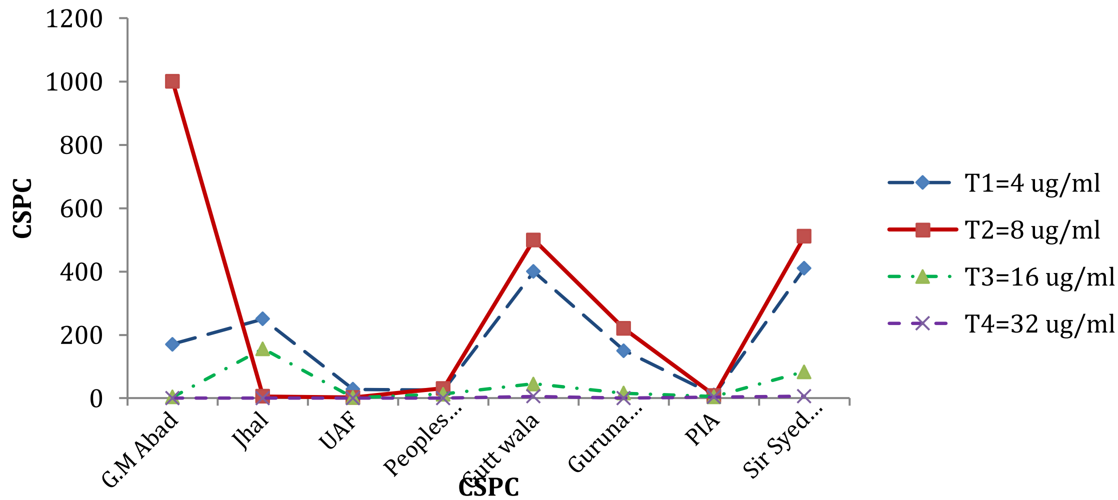
| Gram Staining | | Catalase Test | | Total |
|---|---|---|---|---|
| | | -ve | +ve | |
| **-ve** | Count | 7 | 4 | 11 |
| | % within Gram Staining | 63.60% | 36.40% | 100.00% |
| | % within Catalase Test | 43.80% | 28.60% | 36.70% |
| **+ve** | Count | 9 | 10 | 19 |
| | % within Gram Staining | 47.40% | 52.60% | 100.00% |
| | % within Catalase Test | 56.30% | 71.40% | 63.30% |
| **Total** | Count | 16 | 14 | 30 |
| | % within Gram Staining | 53.30% | 46.70% | 100.00% |
| | % within Catalase Test | 100.00% | 100.00% | 100.00% |

Chi-square value = $0.741^{NS}$
P-value = 0.389
NS = Non-significant (P>0.05)

**A**


1200
1000
800
600
400
200
0
GSK
G.M Abad
Jhal
UAF
Peoples...
Gutt wala
Gurunan...
PIA
Sir Syed...
GSK
T1=4 ug/ml
T2=8 ug/ml
T3=16 ug/ml
T4=32 ug/ml

**B**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1000
800
600
400
200
0
CSPC
G.M Abad
Jhal
UAF
Peoples...
Gutt wala
Guruna...
PIA
Sir Syed...
CSPC
T1=4 ug/ml
T2=8 ug/ml
T3=16 ug/ml
T4=32 ug/ml

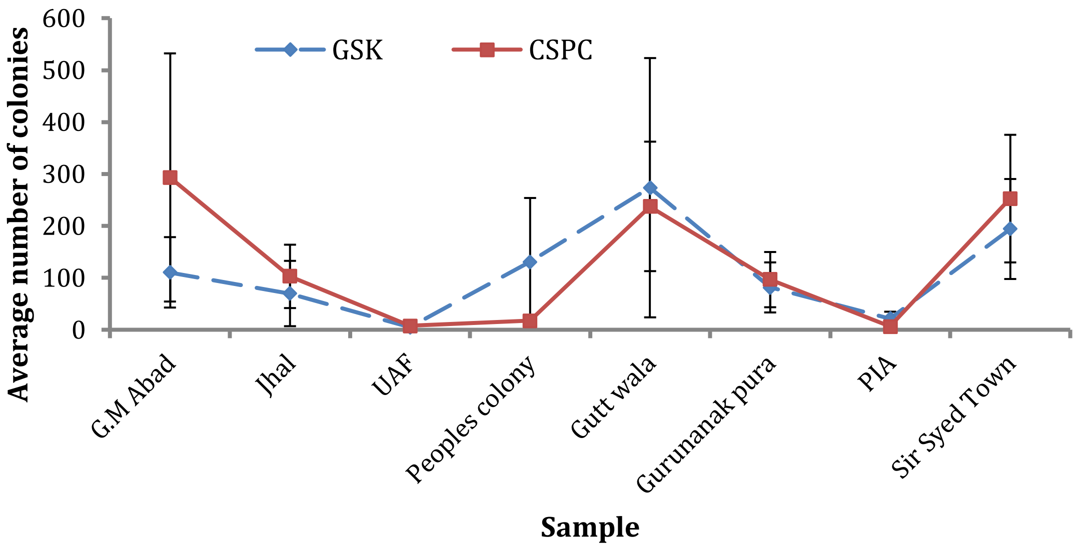
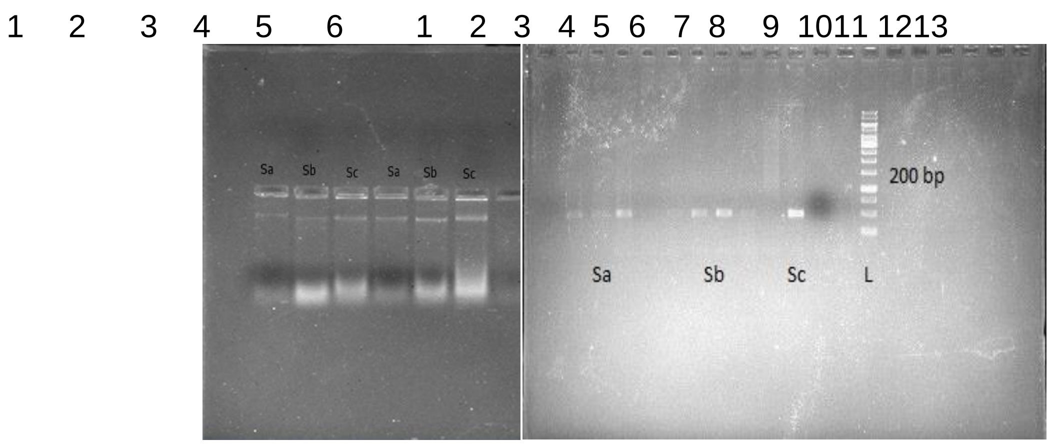



**Fig 1**: Average no. of colonies of resistant bacteria and their comparison **A**: Resistant bacterial colonies against amoxicillin GSK **B**: resistant bacteria against CSPC **C:** graphical representation of a comparison of average no. of colonies against GSK and CSPC




**A** **B**

**Fig 2** Agarose gel electrophoresis analysis of bacterial strains resistant to amoxicillin **A**: Lane 1-6 DNA isolation of resistant bacteria strains of Sa: Streptococcus, Sb: Streptobacillus, and Sc: Staphylococcus. **B**: PCR amplified amoxicillin resistant genes of 130 bp; lane 2-4 amx resistant streptococcus strains, lane 7,8 streptobacillus strains, lane 11 staphylococcus strain