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Phenotypic Methods of Detection of Antibiotic Resistance in Gram Positive Bacteria in Sohag University Hospital

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ABSTRACT

Background: Antimicrobial resistance (AMR) is the ability of a pathogenic microbe to develop a resistance to the effects of an antimicrobial medication. AMR is one of the most concerning issues in medicine today, impacting morbidity, mortality and socioeconomic factors.

Objective: To determine the prevalence and the phenotypic methods of detection of antibiotic sensitivity of gram-positive bacteria in clinical isolates collected from inpatients at the Sohag University Hospital during one year.

Patients and methods: Fresh samples (blood, urine, sputum, pus and cerebrospinal fluid) were collected of 69 patients and cultured on nutrient agar, blood and MacConkey agar, at 37°C and incubated for 24 – 48 hours. The pH was adjusted to 7.4 and all media were sterilized by autoclaving at 121°C for 20 minutes. VITEK2 Compact identification kits was used to confirm the identification of the isolates and for antibiotic susceptibility test

Results: S. aureus were isolated in (43.48%) of isolated organisms and 31.88 % of the cases (n=22) were distributed between other staphylococcus species. S. pneumoniae in 10 cases (14.49%) and 10.15% of the cases (n=7) were distributed between other streptococcus species. All S. aureus isolates were resistant to penicillin but all S. aureus isolates were sensitive to quinupristin/dalfopristin, vancomycin, tigecycline, nitrofurantoin, rifampicin, and trimethoprim/sulfamethoxazole. S. pneumonia showed high rate of resistance to benzylpenicillin (100%) and oxacillin (100%) but the association trimethoprim/sulphamethoxazole showed moderate rate of resistance (50%) to S. pneumonia

Conclusion: The prevalence of antibiotic resistance to gram positive bacteria continues to increase and is associated with significant mortality. The most prevalent organisms within gram positive bacteria were staphylococcus aureus followed by S. pneumoniae.

Keywords: Antibiotic Resistance, Antimicrobial Resistance, Gram Positive Bacteria.

INTRODUCTION

Antimicrobial resistance occurs when microbes evolve mechanisms that protect them from the effects of antimicrobials [(1)]. The term antibiotic resistance is a subset of anti-microbial resistance (AMR), as it applies to bacteria that become resistant to antibiotics. Resistant microbes are more difficult to treat, requiring higher doses, or alternative medications that may prove more toxic. These approaches may also be more expensive. Microbes resistant to multiple antimicrobials are called multidrug-resistant (MDR) [(2)].

Fungi evolve antifungal resistance. Viruses evolve antiviral resistance. Protozoa evolve antiprotozoal resistance, and bacteria evolve antibiotic resistance. Those bacteria that are considered extensively drug resistant (XDR) or totally drug-resistant (TDR) are sometimes called "superbugs"[(3)]. Resistance in bacteria can arise naturally, by genetic mutation, or by one species acquiring resistance from another. Resistance can appear spontaneously because of random mutations. However, extended use of antimicrobials appears to encourage selection for mutations which can render antimicrobials ineffective [(4)].

Rising drug resistance is caused mainly by the use of antimicrobials in humans and other animals, and the spread of resistant strains between the two. Growing resistance has also been linked to the dumping of inadequately treated effluents from the pharmaceutical industry, especially in countries where bulk drugs are manufacture [(5)]. Antibiotics increase selective pressure in bacterial populations, causing vulnerable bacteria to die; this increases the percentage of resistant bacteria, which continue growing. Even at very low levels of antibiotics, resistant bacteria can have a growth advantage and grow faster than vulnerable bacteria [(6)]. With resistance to antibiotics becoming more common there is a greater need for alternative treatments. Calls for new antibiotic therapies have been issued, but new drug development is becoming rare [(7)].

There are public calls for global collective action to address the threat that includes proposals for international treaties on antimicrobial resistance. Worldwide antibiotic resistance is not completely identified, but poorer countries with weaker healthcare systems are more affected [(8)].

The WHO defines antimicrobial resistance as a microorganism's resistance to an antimicrobial drug that was once able to treat an infection by that microorganism [(9)]. A person cannot become resistant to antibiotics. Resistance is a property of the microbe, not a person or other organism infected by a microbe [(10)].

Antibiotic resistance is a subset of antimicrobial resistance. This more specified resistance is linked to pathogenic bacteria and thus broken down into two further subsets, microbiological and clinical. Resistance linked microbiologically is the most common and occurs from genes, mutated or inherited, that allow the bacteria to resist the mechanism associated with certain

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antibiotics. Clinical resistance is shown through the failure of many therapeutic techniques where the bacteria that are normally susceptible to treatment become resistant after surviving the outcome of the treatment. In both cases of acquired resistance, the bacteria can pass the genetic catalyst for resistance through conjugation, transduction, or transformation. This allows the resistance to spread across the same pathogen or even similar bacterial pathogens [(11)].

In 2014 WHO reported that, "This serious threat is no longer a prediction for the future, it is happening right now in every region of the world and has the potential to affect anyone, of any age, in any country. Antibiotic resistance when bacteria change so antibiotics no longer work in people who need them to treat infections is now a major threat to public health"[(12)]. In 2018, WHO considered antibiotic resistance to be one of the biggest threats to global health, food security and development [(13)].

The objectives of this work are to examine antimicrobial resistance in gram-positive bacteria and the rationale for using phenotypic methods in the detection of antimicrobial resistance, their advantages and limitations, to address some of the technical aspects, and then to discuss the application of these techniques for specific purposes.

PATIENTS AND METHODS

This study was a cross sectional laboratory-based study conducted for one year at Sohag University Hospital. This study included 69 isolates from various clinical specimens from patients admitted to different departments in Sohag University Hospital with ages ranged from 6 month to 70 years.

Ethical approval:

This study was approved by the Research and Ethical Committee at the Faculty of Medicine, Sohag University. All subjects were informed about the aim of this study and gave written consents. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Patient data collection:

Fresh samples (blood, urine, sputum, pus and cerebrospinal fluid) were collected soon after collection of data from patients (69 specimens).

Clinical specimens and clinical laboratory work:

All samples were taken under a complete aseptic condition in sterile container according to the standard protocol for each sample. These samples were transported to Microbiology Unit within 2 hours of collection.

Laboratory investigations:

A. Isolation of gram-positive bacteria:

All samples were cultured on nutrient agar, blood and MacConkey agar, at 37°C and incubated for 24 – 48 hours.

Blood samples were cultured on blood culture bottle then put on BACTEC system for more than 5 days. If there were the growth of bacteria in bottle, we cultured it on blood and MacConkey agar.

The following media were prepared according to the manufacturer's instructions or according to as described below. The pH was adjusted to 7.4 and all media were sterilized by autoclaving at 121°C for 20 minutes unless otherwise was mentioned.

B. Identification of Gram-positive bacteria:

1. **Macroscopic appearance of colonies**: On blood agar pale colonies suspect gram positive cocci.
2. **Microscopic examination of Gram-stained colonies:** By gram stain isolates appear as purple-blue cocci.
3. **VITEK2 Compact identification kits was used to confirm the identification of the isolates and for antibiotic susceptibility test** (BioMérieux, Inc. Durham, North Carolina 27704-0969 / USA).

C. Determination of antibiotic susceptibility pattern of isolated organisms:

Antibiotic susceptibility test by VITEK® 2 compact system:

The VITEK® 2 Antimicrobial Susceptibility Tests (AST) are intended for use with the VITEK® 2 Systems for the automated quantitative or qualitative susceptibility testing of isolated colonies.

Statistical analysis

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for the Social Sciences) version 22 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Qualitative data were represented as frequencies and relative percentages. Quantitative data were expressed as mean ± SD (Standard deviation).

RESULTS

Diagnosis:

Table (1) shows that the diagnosis of the studied cases varies. The most common was 20 cases (28.99%) diagnosed as surgical site infection (SSI) and 18 cases (26.09%) were diagnosed as respiratory infection.

Table (1): Distribution of studied cases according to the diagnosis

Diagnosis	N	%
Respiratory infection	18	26.09
GIT infection	6	8.70
SSI	20	28.99
UTI	8	11.59
Pneumonia	2	2.90
Meningitis	1	1.45
Neonatal meningitis	1	1.45
Endocarditis	2	2.90
Sepsis	2	2.90
PUO	3	4.35
Sinusitis	2	2.90
Food poisoning	1	1.45
Dehydration	1	1.45
Fulminant hepatitis	1	1.45
Otitis media	1	1.45
Total	69	100.00

Microbiological results:

Table (2) shows that sputum was the most common specimen in 22 cases (31.88).

Table (2): Distribution of studied cases according to the specimens

Specimens	N	%
Sputum	22	31.88
Blood	10	14.49
Pus	19	27.54
Urine	8	11.59
Stool	8	11.59
CSF	2	2.90
Total	69	100.00

Organism detected:

Table (3) shows that the most common organisms of the studied specimens were S. aureus in 30 cases (43.48%).

Table (3): Distribution of studied cases according to the organism

Organism	N	%
Coagulase negative staphylococcus	1	1.45
Staphylococcus aureus	30	43.48
Staphylococcus hominis	6	8.70
Staphylococcus hemolyticus	3	4.35
Staphylococcus pseudintermedius	5	7.25
Staphylococcus saprophyticus	1	1.45
Staphylococcus xylosus	1	1.45
Staphylococcus schleiferi	1	1.45
Staphylococcus epidermidis	2	2.90
Staphylococcus capitis	1	1.45
Staphylococcus lentus	1	1.45
Streptococcus pneumoniae	10	14.49
Streptococcus agalactiae	1	1.45
Streptococcus gallolyticus	1	1.45
Streptococcus pyogenes	1	1.45
Streptococcus parasanguinis	1	1.45
Streptococcus anginosus	1	1.45
Kocuria rosea	1	1.45
Alloiococcus otitis	1	1.45
Total	69	100.00

Table (4): Number and percentages of studied organisms

Organism	N	%
Staphylococcus aureus	30	43.48
Staphylococci other	22	31.88
Streptococcus pneumonia	10	14.49
Streptococci other	7	10.14
Total	69	100.00

Antibiotic susceptibility:

Table (5) shows antibiotic resistance in the studied case as 100 % were benzylpenicillin resistant. In the studied samples 98.55% were resistant to oxacillin and 1 (1.45) was sensitive. 24.64 % were resistant to gentamycin, 2.90% were intermediate and 72.46 % were sensitive.

Table (5): Distribution of studied cases according to antibiotic susceptibility

	Resistant		Intermediate		Sensitive	
	N	%	N	%	N	%
Benzylpenicillin	69	100.00	0	0.00	0	0.00
Oxacillin	68	98.55	0	0.00	1	1.45
Gentamycin	17	24.64	2	2.90	50	72.46
Ciprofloxacin	20	28.99	2	2.90	47	68.12
Levofloxacin	21	30.43	1	1.45	47	68.12
Moxifloxacin	20	28.99	3	4.35	46	66.67
Erythromycin	44	63.77	3	4.35	22	31.88
Clindamycin	34	49.28	6	8.70	29	42.03
Quinupristin/dalfopristin	16	23.19	4	5.80	49	71.01
Vancomycin	20	28.99	0	0.00	49	71.01
Tetracycline	42	60.87	2	2.90	25	36.23
Tigecycline	18	26.09	0	0.00	51	73.91
Nitrofuratoin	8	11.59	3	4.35	58	84.06
Rifampicin	21	30.43	0	0.00	48	69.57
Trimethoprim/sulfamethoxazole	27	39.13	0	0.00	42	60.87

DISCUSSION

In our study, the age of the studied population ranged from 6 months up to 70 years which is a wide range with mean age ± standard deviation (SD) was 38.099±19.439 years. Regarding the gender, the male to female ratio was 2.1:1. This was similar to the study done by **Licata *et al.*** [14], where the male to female ratio was 1.6:1. Our study is in agreement with other studies which reported that staphylococcus species were the predominant microorganism (40–60%) of the total microorganisms isolated from studied cases [15, 16].

All S. aureus isolates were resistant to penicillin, this agreed with many studies, which reported that more than 80% of isolated S. aureus were resistant to penicillin [17, 18].

In our study S. aureus isolates were moderately resistant to gentamycin (23.33%), erythromycin (46.6%), clindamycin(40%) .These results are in accordance with **Mohammed *et al.*** [19] who found that (34%) of S. aureus isolates were resistant to gentamycin, (34%) to erythromycin (44%) to and clindamycin (38%), with **Belbase *et al.*** [17], who reported that S. aureus isolates were also moderately resistant to gentamycin (31.5%) erythromycin (55.5%) but resistance to clindamycin was lower than our study (11%), and with **Olowo-Okere *et al.*** [20] who reported higher rate of resistance to erythromycin(100%) and gentamycin(85%).

Also S. aureus isolates had higher resistance to tetracycline (76.66%) but **Mohammed *et al.*** [19] reported that S. aureus isolates were moderately resistant to tetracycline (54%) [21].

S. aureus isolates showed similar resistance pattern to ciprofloxacin and levofloxacin (20%) to moxifloxacin (4%). These results are in agreement with a study performed by **Mohammed *et al.*** [19], where S. aureus isolates showed similar resistance pattern to ciprofloxacin and levofloxacin (26%) and **Onwubiko and Sadiq**[22] who reported that 31% of isolated S. aureus were resistant to ciprofloxacin.

All S. aureus isolates were sensitive to qunipristin/dalfopristin, vancomycin, tigecycline, nitrofurantoin, rifampicin, and trimethoprim/sulfamethoxazole. Most studies reported no or low resistance of S. aureus of to these antibiotics such as the studies performed by **Belbase *et al.*** [17], **Neopane *et al.*** [18] and **Mohammed *et al.*** [19]. Low resistance of S. aureus isolates has been reported by **Bukhari *et al.*** [23] to vancomycin (1%), nitrofuratoin (4.2%), and rifampicin (2%) and results of a study of **Gitau *et al.*** [24] were consistent with our results as they reported (2%) resistance to nitrofuratoin and tigercycline, 5% to vancomycin and (8%) to rifampicin. **Roy *et al.*** [21] and **Rahim *et al.*** [25] reported higher resistance rate to vancomycin (24.1%) and (26.6%) respectively.

The results of this test showed that S. pneumonia has a great resistance to most commonly antibiotics used in hospitals, the highest rate of resistance is seen with benzylpenicillin (100%) and oxacillin (100%) but **Engelbrecht *et al.*** [26] reported moderate resistance to benzylpenicillin (45.9%).

In this study S. pneumonia showed high resistance to erythromycin (80%) clindamycin (50%) gentamycin (50%) trimethoprim/sulfamethoxazole (50%) but it showed moderate resistance to ciprofloxacin and levofloxacin (40%) rifampicin (30%). S. pneumonia showed low resistance to moxifloxacin (20%) and tetracycline nitrofurantoin (20%) but showed high sensitivity to vancomycin, tigecycline and quinupristin/dalfopristin [27].

The highest rate of resistance was seen with erythromycin 37/37 (100%), azithromycin 31/37 (83.8%), clindamycin 31/37 (83.8%) and trimethoprim/sulfamethoxazole 30/37 (81.1%) and moderate resistance to tetracycline 22/37 (59.5%) and benzylpenicillin17/37 (45.9%), whereas relative lower resistance was seen towards levofloxacin 13/37 (35.1%), ciprofloxacin 6/37 (16.2%), gentamicin 4/37 (10.8%), oxacillin 3/37 (8.1%), and rifampicin 3/37 (8.1%). Development of antibiotic resistance is often related to the overuse and misuse of the antibiotics prescribed. Resistance of S. pneumoniae continues to be an important clinical therapeutic problem, such that there is an increasing multidrug resistance in these bacteria. The results of this study showed that S. pneumoniae isolates were found to be remarkably sensitive to vancomycin (100%) [27]. **Flamm *et al.*** [28] found that S. pneumonia was evaluated for MDR status against penicillin, ciprofloxacin, erythromycin, tetracycline (TET), trimethoprim/ sulfamethoxazole (TMP/SMX), and levofloxacin (LEV). MDR were defined as non-susceptible (NS) to at least 2 of the above agents. There was a high rate of resistance to erythromycin at 42.7%, and resistance to tetracycline, trimethoprim/sulfamethoxazole and clindamycin ranged from 20.3 to 24.6%. The results of the current study disagree with the **Flamm *et al.*** [28] study since, the S. pneumoniae isolates in this study exhibited a low rate of susceptibility to levofloxacin and other antibiotics, while showing 100% resistance to erythromycin.

In Lebanon, S. pneumoniae isolates have shown increasing resistance to penicillin, macrolides, and other anti-microbial agents as in our study [29]. **Rijal *et al.*** [30] have pointed out that 2.17% of S. pneumoniae isolates were resistant to erythromycin. This result was inimputable with results of the study in which 100% of isolates appeared to be resistant to erythromycin.

The association trimethoprim/sulphamethoxazole used in this study showed moderate rate of resistance (50%) but the study of **Motaweq and Naher** [27] showed high rate of resistance, 81% full resistance. Compared with a preceding study with strains in Brazil, there was an increased number of resistant strains, similar to the situation in other countries [31]. Results of our study of tetracycline resistance (20%) is close to study by **Levin *et al.*** [32] who observed a rate of tetracycline resistance (32%). In our study, other streptococcus species were highly resistant to benzylpenicillins (100%) and highly resistant to oxacillin (100%). That is close to **Süzük *et al.*** [33] study on viridin streptococcus, which found the rates of resistance and reduced sensitivity of the isolates for penicillin and ampicillin were determined at 61.2% and 55.1%, respectively.

In our study, (42.8%) susceptible to tetracycline (85.7%) were considered susceptible to nitrofurantoin, (57.1%) to rifampicin (71.4%) to trimethoprim-sulfamethoxazole and that goes in line with the result of **Quiroga *et al.*** [34] in which 29.0% were susceptible to tetracycline, 98.3% of the isolates were considered susceptible to nitrofurantoin, 96.8% to rifampicin, and 46.8% to trimethoprim- sulfamethoxazole. In our study, (57.1%) were resistant to clindamycin and (85.7%) were resistant to erythromycin. But in contrast **Quiroga *et al.*** [34] found that 7 cases (3.2%) were resistant to clindamycin and 6 cases (9.7%) were resistant to erythromycin. In our study (85.7%) were sensitive to gentamycin in contrast to **Quiroga *et al.*** [34] who found high resistance to gentamycin.

CONCLUSIONS

- The prevalence of antibiotic resistance to gram positive bacteria continues to increase and is associated with significant mortality**.**
- The most prevalent organisms within gram positive bacteria were staphylococcus aureus followed by S. pneumoniae.
- The commonest type of infection was associated with pulmonary tract infection.
- According to antibiotic susceptibility there was high resistance to benzylpenicillin (100%) in the studied samples 98.55% were resistant to oxacillin, 24.64 % were resistant to gentamycin**.**
- 28.99% of samples were also resistant to ciprofloxacin, 30.43% of samples were resistant to levofloxacin, 28.99% were resistant to moxifloxacin and 63.77 % were resistant to erythromycin**.**
- There were 16 (23.19%**)** cases resistant to quinupristin/dalfopristin.
- In the studied cases, 20 cases (28.99 %) were resistant to vancomycin. 42 cases (60.87%) were resistant to tetracycline. 18 cases (26.09%**)** were resistant to tigecycline.
- 11.59% of samples were resistant also to nitrofuratoin, 30.43 were resistant to rifampicin and 39.13% were resistant to trimethoprim/sulfamethoxazole.

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