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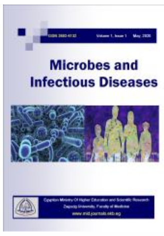

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Original article

The activity of some dyes against clinically isolated bacteria

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ABSTRACT

Background: The increasing resistance of many bacterial pathogens against antibiotic demanded urgent new or repurposing therapeutic strategies such as utilizing certain dyes that may be a promising branch in microbial therapy. **Material and methods**: Two types of bacteria, *Escherichia coli* (*E. coli*) were isolated from the urine sample and *Staphylococcus aureus* (*S. aureus*) from the wounds using cotton swabs. The well diffusion method was used after overnight incubation for antibiotic sensitivity testing. The diameter of inhibited growth was measured per millimeter for three antibiotics (amoxicillin, gentamicin, ceftriaxone) that were added to one dish, and dyes (leishman stain1, leishman stain 2, India ink, crystal violet, safranin) that were added to the other dish. **Results**: Crystal violet was 100% active against *E. coli* and 50% active against S. *aureus*. Safranin was 25% active against *E. coli* while it showed higher activity against *S. aureus* 75%. India ink and Leishman stain 2 were inactive against *S. aureus* whereas they revealed 50% and 25% activity against *E. coli* respectively. Leishman stain 1 was 25% active against *E. coli* and 50 % active against *S. aureus*. The mean of inhibition zone of amoxicillin in *E. coli* 40.75± 11.95 mm that was higher than *S. aureus* 35.50±6.40 mm without significant differences (**P**= 0.46). The other antibiotics, gentamicin and ceftriaxone, also showed no significant differences (**P**= 0.29 and **P**= 0.85) respectively. The mean of inhibition zone of Crystal violet in *E. coli* 20.25± 0.5 mm that was higher than *S. aureus* 17.25± 13.88 without significant differences (**P**= 0.68). **Conclusion**: All investigated dyes (leishman1, leishman2, India ink, crystal violet, safranin) showed antibacterial activity in different percentages. Crystal violet was 100% sensitive to *E. coli* and 50% sensitive to *S. aureus*.

Introduction

Dyes are compounds that are commonly used to impart color to various materials, including fabrics, cosmetics, and food products. However, some dyes have been found to possess antibacterial properties and can be used as potential agents for controlling bacterial infections [1].

Leishmania stain and India ink are two different stains used in microbiology for visualizing certain types of microorganisms [2]. It is a differential staining technique used for identifying Leishmania parasites, India ink is a negative staining technique used for visualizing capsules of certain microorganisms. Both stains are useful tools in microbiology for diagnosing specific types of infections [3].

Safranin is a biological stain used in histology and microbiology to differentiate and visualize certain structures, such as cell walls, cytoplasm, and nuclei, under a microscope. It is a basic dye that stains acidic structures, such as the cell walls of plant and bacterial cells, red or pink [4].

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Staphylococcus aureus is a major pathogen of increasing importance due to the rise in antibiotic resistance [5]. The species named aureus refers to the fact that colonies (often) have a golden color when grown on solid media [6].

E. coli is a Gram-negative, non-sporulating, rod-shaped, facultative anaerobic and coliform bacterium pertaining to the genus *Escherichia* that commonly inhabits the environment, foods, and warm-blooded animals' lower gut [7-9].

Gentamicin is an antibiotic commonly used to treat infections caused by *E. coli* [10-11]. However, the resistance of *E. coli* to gentamicin has been increasing in recent years. A study found that the resistance of *E. coli* to gentamicin was as high as 45.7% in some regions of India [12].

Aminoglycosides (AGs) have been used for decades as effective agents against most Gram-negative pathogens including *Escherichia coli, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa* [13-14].

Ceftriaxone is a beta-lactam, third-generation cephalosporin antibiotic with bactericidal activity. It binds to and inactivates penicillin-binding proteins (PBP) located on the inner membrane of the bacterial cell wall. PBPs participate in the terminal stages of assembling the bacterial cell wall, and in reshaping the cell wall during cell division. Inactivation of PBPs interferes with the cross-linkage of peptidoglycan chains necessary for bacterial cell wall strength and rigidity. This results in the weakening of the bacterial cell wall and causes cell lysis [15].

Urinary tract infections (UTIs) are the most common bacterial infection requiring medical care [16-17]. Over 10.8 million patients in the United States visited the emergency department for the treatment of UTIs between 2006 and 2009 and 1.8 million patients (16.7%) were admitted to acute care hospitals [18]. The economic burden for the treatment of UTIs is estimated at $2 billion annually. In addition, UTIs rank as the number one infection that leads to an antibiotic prescription [19-20].

The aims of the study were detection the effect of gentamicin, ceftriaxone, and amoxicillin on the growth of *E. coli* and *S. aureus*. As well as evaluation of some dyes (leishman stain1, leishman stain 2, India ink, crystal violet, safranin) as antimicrobial agents against isolated *E. coli* and *S. aureus.*

Material and methods

A total number of eight positive culture patients (subjects) were enrolled in this study during the period 5/8/2022 to 8/1/2023.These patients from the urology department, each patient suffering from complaint of frequent urge to urinate and painful, burning feeling in the bladder or urethra during urination. As well as, patients suffering from wound infections. The number and date of projects approval were 8492 in August 01, 2022.

To reduce the risk of contamination, participants were informed to clean their hands with water and their genital area with swab soaked in normal saline before collection of the clean catch mid-stream urine samples. After the urethra is properly cleaned, the collection began by discarding the initial stream of urine into the toilet. Then, 10-15 milliliters (ml) of urine were collected in the provided sterile specimen cup by directly urinating into the cup. Once an adequate amount was collected, the remaining urine was voided in the toilet. For men, the opening of the urethra (tip of the penis) was wiped clean with a cleansing wipe before the collection began. The collected urine samples were analyzed soon within 1 hour after collection.

All isolates were identified based on their morphology, Gram staining, biochemical tests, and culture on selective media (EMB agar) for *E. coli* and (Mannitol salt agar) for *Staph* species. The isolates were identified at first by standard microbiological and biochemical tests [21].

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. The bacterial suspension was adjusted to match the turbidity of a (0.5) McFarland standard. The agar plate surface was inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 mm was punched aseptically with a sterile cork borer or a tip, and a volume (50 µL) of the antimicrobial agent or dye solution at desired concentration was introduced into the well. Then, agar plates were incubated under suitable conditions depending upon the tested microorganism. The antimicrobial agent diffused in the agar medium and inhibited the growth of the microbial strain tested [22].

All the statistical analysis was done by using SPSS 26 software and Excel app. For statistical analysis, SPSS software 26 (SPSS Inc., Chicago, USA) was used. Means and standard deviations were used to represent the data. For correlation analysis, Spearman's correlation for non-parametric analysis was used. Chi- square was used for non- parametric variables. **P** value < 0.05 was taken into account to denote statistical significance additionally.

Results

From the total of eight isolates, four bacteria were *E. coli* and others were *S. aureus*. Females 62% were more prevalent than males 32%.

Crystal violet was 100% active against *E. coli* and 50% active against *S. aureus*. Safranin was 25% active against *E. coli* while it showed higher activity *S. aureus* 75%. India ink and Leishman stain 2 were inactive against *S. aureus* whereas they revealed 50% and 25% activity against *E. coli* respectively. Leishman stain 1 was 25% active against *E. coli* and 50 % active against *S. aureus*. **(Table 1)** and **(Figure 1)**.

Table 1. The percentage of antimicrobial sensitivity of bacteria

Name of bacteria	Leishman stain 1	Leishman stain 2	India ink stain	Crystal violet	Safranin stain
E. coli	25 %	25 %	50 %	100 %	25%
Staph. aureus	50 %	0 %	0 %	50 %	75%

Table 2. Comparison means of inhibition zone of different antibiotics between both bacteria

Agents	Mean of inhibition zone	Std. Deviation	P value
Amoxicillin			
E. coli	40.75	11.955	0.46
S. aureus	35.50	6.403	
Gentamicin			
E. coli	37.75	3.862	0.29
S. aureus	42.00	6.325	
Ceftriaxone			
E. coli	47.25	7.136	0.85
S. aureus	48.00	2.828	

The mean of inhibition zone of Crystal violet for *E. coli* was 20.25±0.5 mm that was higher than *S. aureus* 17.25± 13.88 without significant differences (**P**= 0.68) **(Table 3)**

Table 3: Comparison means of inhibition zone of crystal violet between both bacteria

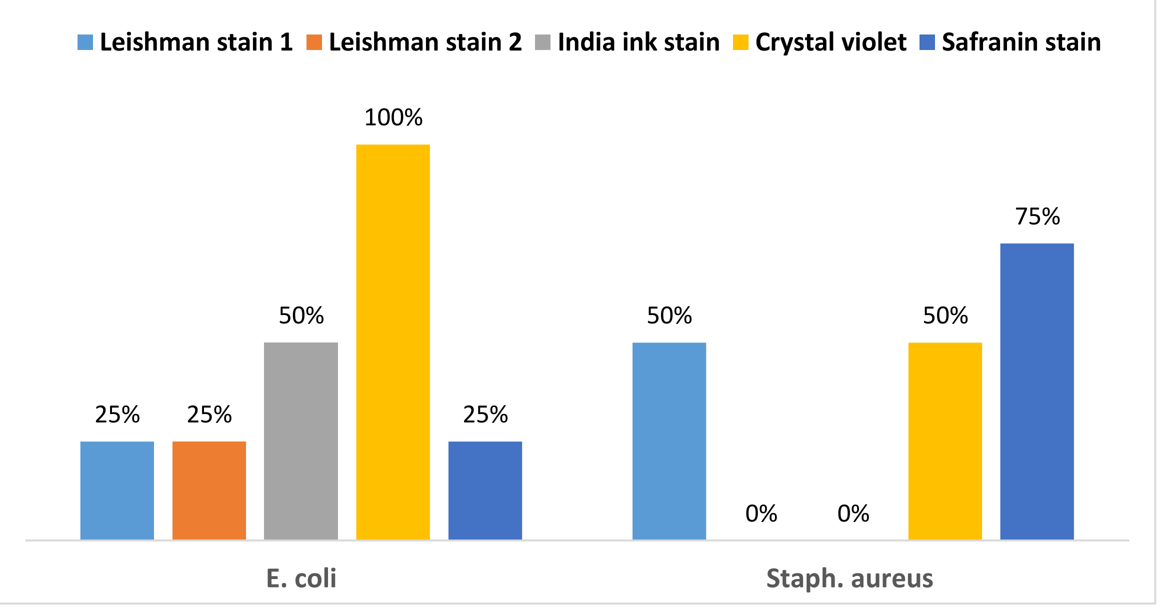
Agents	Mean of inhibition zone	Std. Deviation	P value
Crystal violet			
E. coli	20.25	0.50	0.68
S. aureus	17.25	13.88	

No correlation observed among different agents (**P**> 0.05)

Table 4: Correlation between different agents

Variables		Gentamicin	Amoxicillin
Ceftriaxone	Pearson Correlation	-.134-	.637
	Sig. (2-tailed)	.751	.089
Gentamicin	Pearson Correlation		-.237-
	Sig. (2-tailed)		.571

Figure 1. The percentage of sensitive bacteria to different agents



The mean of inhibition zone of amoxicillin in *E. coli* 40.75± 11.95 mm that was higher than *S. aureus* 35.50± 6.40 mm without significant differences (**P**= 0.46). Other antibiotic gentamicin and ceftriaxone also showed no significant differences (**P**= 0.29 and **P**= 0.85) respectively. **(Table 2)**

Discussion

The comparison of mean inhibition zone of different agents is an important aspect of antimicrobial susceptibility testing. The inhibition zone is the clear area surrounding a disc containing an antibiotic or other antimicrobial agents, which indicates the extent to which the agent can inhibit the growth of a particular microorganism [23]. Overall, the comparison of mean inhibition zone of different agents can provide valuable information for clinicians and researchers in selecting the most effective antimicrobial agents for treating specific infections. There have been many studies comparing the mean inhibition zone of different antimicrobial agents against various microorganisms [24].

This study showed that Urinary tract infection (UTI) in females 62% was more prevalent than males 32%, because women have short and wider female urethra and bacteria can travel from the anus to the urethra [25]. Furthermore, women lack the bacteriostatic properties of prostatic secretions [26-27].

Urinary tract infection is less common in men than in women because the male urethra is long, making it difficult for bacteria to spread to the bladder. Women are more prone to UTIs than men because the urethra is much closer to the anus and is shorter than in males; furthermore, women lack the bacteriostatic properties of prostatic secretions [28]. Among the elderly, UTI frequency is roughly equal in women and men. This is due, in part, to an enlarged prostate in older men. As the gland grows, it obstructs the urethra, leading to increased frequency of urinary retention [29].

In the current study, Crystal violet was 100% sensitive to *E. coli* and 50% sensitive to *S. aureus.* Safranin was 25% sensitive to *E. coli* while it showed higher sensitivity for *S. aureus* 75%. India ink and Leishman stain 2 were 100 resistant to *S. aureus.*

One study that investigated the sensitivity of *E. coli* to safranin found that the bacteria were able to take up the dye even at very low concentrations, suggesting that safranin may be a useful tool for staining *E. coli* cells in microscopy studies [30]. One study that used safranin stain is "Quantitative assessment of bone healing by safranin O staining and micro-computed tomography [31].

Another study mentioned that crystal violet dye has been shown to be 100% sensitive to both Gram-positive and Gram-negative bacterial isolates. Although safranin also had high sensitivity (100%) to *S. aureus* isolates, its sensitivity to *P. aeruginosa* was only 20%. *Staphylococcus aureus* was more resistant to iodine (40% sensitivity) compared to *P. aeruginosa*, which was 100% sensitive to iodine [32].

In the current study, The mean of inhibition zone of amoxicillin in *E. coli* 40.75± 11.95 mm that was higher than *S. aureus* 35.50± 6.40 mm without significant differences (**P**= 0.46). Other antibiotics; gentamicin and ceftriaxone also showed no significant differences (**P**= 0.29 and **P**= 0.85) respectively.

Many studies revealed high sensitivity of *E. coli* to gentamicin [33-34]. Another study mentioned that the resistance of *E. coli* to gentamicin was as high as 45.7% in some regions of India [35]. Another study reported a similar trend of increasing resistance of *E. coli* to gentamicin in hospitals in Iran [36]. On the other hand, some studies have reported a lower level of resistance of *E. coli* to Gentamicin. For example, a study found that only 4.4% of *E. coli* isolates from a hospital in Brazil were resistant to Gentamicin [32].

Conclusion

Overall, the study raises intriguing possibilities for the use of dyes as antimicrobial agents and highlights the need for further research in this area.

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