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## Molecular Detection of Asymptomatic Bacteriuria and its bacterial phages in adolescents in Mosul City / Iraq

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### ABSTRACT:

Asymptomatic Bacteriuria (ASB) is a pathological state distinguished by substantial urinary infection in the absence of any observable symptoms. ASB is a result of the presence of typical gut microorganisms that migrate via the urethra and potentially reach the bladder and kidneys. The diagnosis relies on urine culture, genetic analysis using The Polymerase Chain Reaction (PCR), and bacteriophage administration as a therapeutic intervention. **Methods:** The examination was conducted as a prospective study at Mosul Laboratories. The study lasted for one month, A total of 100 participants, aged between 15 and 33, were recruited, including representatives from both sexes. All individuals involved in the study were clinically verified to display no indications or manifestations of Urinary Tract Infection (UTI).

**Results:** The study involved 100 volunteers and cultured urine samples. Bacterial isolates were identified using the Vitek-2 System and PCR methods. Bacteria prevalence was 84%, higher among 15-20 year olds. GPB species were more prevalent in females, with 61% of isolated cases being Gram-Positive Bacteria (GPB) and 23% being Gram-Negative Bacteria (GNB). which was utilized to do sensitivity testing on different antibiotics utilizing Antimicrobial Susceptibility Testing (AST) cards. Identified species included *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.

**Conclusion:** Non-pregnant women and healthy males frequently experience ASB. Regular urine culture sensitivity testing should be finished for people of all ages corporations and sexes to mitigate the ability dangers and outcomes connected with this condition.

**Keywords:** Asymptomatic bacteriuria, urine, age, sex, phage, PCR and genes

### INTRODUCTION

Asymptomatic bacteriuria (ASB) refers to the presence of a widespread range of microorganisms in a fantastic urine subculture. Most persons with silent bacteriuria are cross-asymptomatic and do not

experience any positive results from antibiotic therapy. According to [1,2], the urinary tract generally keeps sterility because of the physical and chemical properties of urine and the innate immune system's action. Regardless of the presence of

pyuria, aseptic syncytial bladder (ASB) is defined by the presence of 1 or extra bacterial species in the urine at precise quantitative stages (one zero five Colony-Forming Units CFU/mL or 10<sup>8</sup> CFU/L) without any symptoms or signs associated with urinary tract infection (UTI). The situation is located in certain stable lady populations in addition to a substantial number of ladies and men who have abnormalities in their genitourinary tract that hinder regular voiding or are prompted by the regular gut microbiota. These abnormalities in the end migrate through the urethra and potentially affect the bladder and kidneys [3]. *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* are the maximum standard causative organisms. *Proteus mirabilis* is the most commonly encountered organism among institutionalized patients. Patients with long-term urinary catheter use often present with polymicrobial bacteriuria, which includes *Pseudomonas aeruginosa*, *Morganella morganii*, and *Providencia stuartii*. This bacteriuria is also on an upward march. *Enterococcus faecalis*, coagulase-terrible *Staphylococcus*, and Institution B *Streptococcus* are examples of Gramme-high-quality organisms [4-6]. The prevalence of this condition is believed to be 3-5% among younger wholesome women, with a higher incidence observed in patients with diabetes. There appears to be a correlation between the elderly and destructive effects within some demographic agencies, inclusive of the incidence of pyelonephritis or premature birth among pregnant women. The administration of AB to pregnant women has exhibited a decrease in the incidence of pyelonephritis and infants with low birth weight. Despite the lack of advice, the remedy for AB has been found to provide unsatisfactory effects and may result in the emergence of multidrug-resistant bacteria [7]. The occurrence of symptomatic urinary tract infections (UTIs) was elevated in the next screening and long-term follow-up programs for Antibiotic Resistance (AB) among female college students and women. The best corporations who always did not see any benefits from the antibiotic

remedy of AB in prospective, randomized, comparative trials were premenopausal women, children, the elderly, sufferers with lengthy-term catheters, and diabetic patients. An area of ongoing research includes the utilization of notably pathogenic stress of *Escherichia coli* to set off antibiotic resistance (AB) as a therapeutic intervention aimed at preventing symptomatic urinary tract infections (UTIs) in some humans [8]. Certain populations in underdeveloped international locations may additionally show accelerated AB occurrence, although extra research is vital to clarify the underlying factors contributing to this phenomenon. Antimicrobial prophylaxis refers to the management of antibiotics as a safety measure in such cases. Nevertheless, counseling might not continuously yield positive effects for a few individuals [9]. Antibiotic abuse has been a substantial hassle in recent years, resulting in improved healthcare prices and an upward push towards antimicrobial resistance [10]. Phages have been used as an opportunity for antibiotics in France, the former Soviet Union, and Central Europe since the latter part of the twentieth century. Phages of this nature are available as a prospective therapeutic method for pathogens that have obtained resistance from several pharmaceutical sellers. Phages have the potential to interact with immune devices both at once through immunity and circuitously through the era of phage-encoded proteins through microorganisms [11]. Phage therapy has numerous advantages over antibiotic healing procedures because of its high degree of host specificity. The comparison between bactericidal and bacteriostatic marketers resulted in increased awareness within the host, which probably necessitated a single healing dose. The biosphere includes widespread quantities of low intrinsic toxicity. Multiple phages can be blended in a cocktail to selectively target multiple bacteria simultaneously. The administration of the substance can be presented in various forms, including liquid, powder, ointment, drugs, and so on [12]. There is a need for a phage cocktail that is more

efficient and powerful and that has a broader range of motion because most diseases are a result of multiple types of microorganisms (Divya Ganeshan and Hosseinidoust, 2019). Concerns over phage resistance and the effect of phages on bacterial genome evolution have hindered the approval of the FDA and EMA. Nevertheless, phages can invade microorganisms even when they are in a kingdom of inactivity. Other traits, including enzymes, resistance to serum inactivation, mutation rate, and resistance to contamination, were observed to be important and require additional research [13]. This study aimed to examine the superiority of asymptomatic bacteriuria in non-pregnant females and healthy adult males, as well as to determine the capability of bacteriophages as a therapeutic approach.

#### **MATERIALS AND METHODS:**

This prospective study was performed in the laboratories of Mosul Hospital, Mosul, in Mosul City. The investigation extended over one month, specifically, to May 2021. Before their inclusion in the look-at, all participants provided informed consent. The examined included non-pregnant females and healthy males who did not exhibit any indicators or signs of urinary tract infection. The contributors have been given instructions to post a midstream urine specimen that was considered “a smooth trap” if they want to minimize the danger of infection. In addition, they were advised to cleanse their fingers before accumulating urine samples.

#### **Collection Of Samples:**

The samples were gathered in aseptic containers and examined within a timeframe of 2 h from the time of collection. The samples were cultivated on blood, MacConkey, and nutrition agar, following the

manufacturer’s recommendations for preparation. The cultures were then incubated at a temperature of 37°C for 24 h. Any samples revealing pollutants were disposed of a urine culture was considered to indicate significant asymptomatic bacteriuria (ASB) if the count was above 100,000 colony-forming units per milliliter (>100,000 cfu/ml). The bacterial colonies were isolated and classified according to their morphology, pigmentation, and dimensions. Biochemical tests and an active pharmaceutical ingredient (API) system were used to validate the diagnosis. The Vitek-2 system was also used. The Vitek2 system was used to do the sensitivity testing. The Zippy™ Plasmid Miniprep Kit (product from ZYMO RESEARCH / USA, serial number D4036) was used to extract plasmid DNA samples from genomic plasmids to diagnose resistance genes present on plasmids. PCR technology was used to accomplish this task. The primers listed in Table (1) were developed by the Integrated DNA Technologies Company, Canada. A stock solution of each primer at a concentration of 100 pmol/μl was prepared by reconstituting lyophilized powder form with distilled and sterile water. The resulting stock solution was then stored at 20°C. The storage solution was prepared by combining 10 μl of the primer solution with 90 μl of distilled water, resulting in a final volume of 100 μl.

#### **Isolation and Determination of Bacteriophage Titre:**

A sterile sample of the hospital’s sewage water was filtered through a 125-mm sterile and dry filter paper to remove any attached materials and impurities and reduce pollution. Then, the method described by [14].

The number of bacteria in (1) ml of the initial water sample was determined using the following equation.

$$\text{Number of plaque bacteria} = \frac{\text{Number of areas analyzed per ml}}{\text{Dilution} \times \text{Quantity dilution Required}} [15].$$

Table ( 1 )Sequence of primers used to investigate resistance genes.

The primer name	Sequence of nitrogen bases '5 -'3	Size of the target gene (bp)
<i>acc(3)-II – F,</i> <i>acc(3)-II – R,</i>	5'- TGAAACGCTGACGGAGCCTC- 3'	370
	5'- GTCGAACAGGTAGCACTGAG - 3'	
<i>drf – F</i> <i>drf – R</i>	5'- TCATGGCTTGTATGACTGT- 3'	1980
	5'- GTAGGGCTTATTATGCACGC- 3'	
<i>bla<sub>SMY-2</sub> – F</i> <i>bla<sub>SMY-2</sub> – R</i>	5'- GCGGTGTTGGGCGGCGATG - 3'	369
	5'- CAGCGGAACCGTAATCCAG- 3'	

### Statistical Analysis:

The SWSS software package version 23 (IBM, USA) was used to analyze the findings of the present investigation. The statistical data analysis involved the use of chi-square and percentage tests and personal tests.

### RESULTS:

The study revealed that the incidence of AB was 84%, with the highest incidence observed in females and males aged between (15 – 20) years, at 38.1% and 11.9%, respectively, as in Table (2)

Females had a greater incidence of AB than males, with 70.2% of females and 29.8% of males testing positive for culture, as shown in Table 2.

A notable increase in Gram-positive bacteria was identified in 84% of the 100 processed samples, while Gram-negative bacteria constituted 16% of the cases. Table 3 presents the data for Gram-positive bacteria, indicating that the prevalence was 16% in males and 45% in females. For Gram-negative bacteria were detected in 9% of males and 14% of females. In the first age group (15-20 years), the distributions of total Gramme-positive and Gramme-negative bacteria were 34.5% and 15.5%,

respectively. as indicated in Table (3)

Plasmids were extracted from gram-negative and gram-positive bacterial strains, and the genes *acc(3)-II*, *dr*, and *blacmy-2* were identified. The *acc(3)-II* gene confers resistance to aminoglycoside like gentamicin. These genes were detected in strains of gram-negative bacteria: *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Enterobacter cloacae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*. It was also found in one strain of gram-positive *Staphylococcus aureus*. The gene *drf*, which is responsible for resistance to trimethoprim and sulfamethoxazole, was observed in three strains: *Klebsiella pneumoniae*, *Escherichia coli*, and *Acinetobacter baumannii*. The *black-2* gene, which is associated with resistance to ceftriaxone, is present in only two strains: *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. These findings are depicted in Figures (1, 2, 3, and 4).

Figure (5) depicts the identification of bacteriophages against specific strains. The specific bacteriophage exhibited a good response to the strain obtained from the hospital samples. When comparing the findings of [16], a certain degree of consistency was observed, as demonstrated in the study conducted by Figer (5).

**Table 2: Number and percentage of patients with asymptomatic Bacteriuria (AB) According to the Age group(years) and Sex.**

Age (years) \ Sex No. (%)	15-20	21-26	27-32	>33	Total
Males 25(29.8)	10(11.9)	7(8.3)	4(4.8)	4(4.8)	25(29.8)
Females 59(70.2)	32(38.1)	11(13.1)	8(9.5)	8(9.5)	59(70.2)
Total 84(100)	42(50)	18(21.4)	12(14.3)	12(14.3)	84(100)

**Table 3: Number and Percentage of bacteria in different populations with Asymptomatic Bacteriuria.**

Bacteria \ Sex No(%)	Gramme Positive	Gramme Negative	Sterile
Males 30(30)	16(16)	9(9)	5(5)
Females 70(70)	45(45)	14(14)	11(11)
Total 100(100)	61(61)	23(23)	16(16)

**Table 4: Percentage and Number of Bacteria in Asymptomatic Bacteriuria (AB) According to the Age group(years).**

Bacteria \ Age(years)	15-20	21-26	27-32	>33
Gramme Positive 61(72.6)	29(34.5)	12(14.3)	12(14.3)	8(9.5)
Gramme Negative 23(27.4)	13(15.5)	6(7.1)	0(0)	4(4.8)
Total 84(100)	43(50)	18(21.4)	12(14.3)	12(14.3)

Table 5 Number and Percentage of Bacterial species under study.

Gramme +Bacterial Species	No.	Gramme +Bacterial species	No.
<i>Staphylococcus hemolytic</i>	9	<i>Enterococcus faecalis</i>	10
		<i>Micrococcus luteus</i>	3
<i>Staphylococcus hominis</i>	8	<i>Brevibacterium spp.</i>	2
<i>Staphylococcus aureus</i>	12		
<i>Staphylococcus epidermidis</i>	5		
<i>Staphylococcus saprophyticus</i>	2	<i>Streptococcus agalactiae</i>	1
<i>Staphylococcus auricularis</i>	2	<i>Enterococcus avium</i>	1
<i>Staphylococcus warneri</i>	1	<b>Total</b>	<b>56</b>
Gramme -Bacterial Specie	No.	Gram-Bacterial Species	No.
<i>Escherichia coli</i>	15	<i>Pseudomonas aeruginosa</i>	6
<i>Klebsiella pneumoniae</i>	18	<i>Proteus mirabilis</i>	5
<i>Acinetobacter baumannii</i>	9	<b>Total</b>	<b>53</b>

Table 6: Susceptibility of G-ve bacterial species to antibiotics by Vitek -2.

Species Name	Antibiotics	Resistant of Gramme-negative bacterial Species				
		Phylum Proteobacteria				
		<i>E. coli</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	<i>Acinetobacter baumannii</i>	<i>Proteus Mirabilis</i>
Penicillin	Ticarcillin	5	4	3	2	2
	Azlocillin	4	3	3	2	2
	Piperacillin	5	4	3	2	2
Beta-lactam combination	Piperacillin/tazobactem	4	3	3	2	2
Monobactams	Aztreonam	5	5	3	2	2
Cephalosporin	Cefazolin,	4	5	3	2	2
	Ceftriaxone	4	5	2	1	1
	Ceftazidime	4	3	3	2	2
	Nitrofurantoin	4	3	2	2	2
Fluoroquinolone	Nalidixic acid	5	4	3	2	2
	Fleroxacin	5	3	0	1	2
	Levofloxacin	4	1	0	1	2
	Moxifloxacin	4	2	0	1	2
	Sparfloxacin	4	2	0	1	2
	Ciprofloxacin	4	3	2	1	2
Carbapenems	Doripenem	3	4	3	2	2
	Etrapenem	3	4	1	2	2
	Imipenem	2	1	1	0	0
	Meropenem	2	1	1	0	1
Aminoglycoside	Amikacin	4	0	0	0	0
	Gentamicin	3	1	0	0	0
	Netlimicin	4	1	0	1	2
	Tobramycin	4	1	0	1	1
Tetracycline	Minocycline Tetracycline	4	1	2	1	2
Sulphonamides	Trimethoprim/ Sulfamethoxazole	14	3	3	2	2)

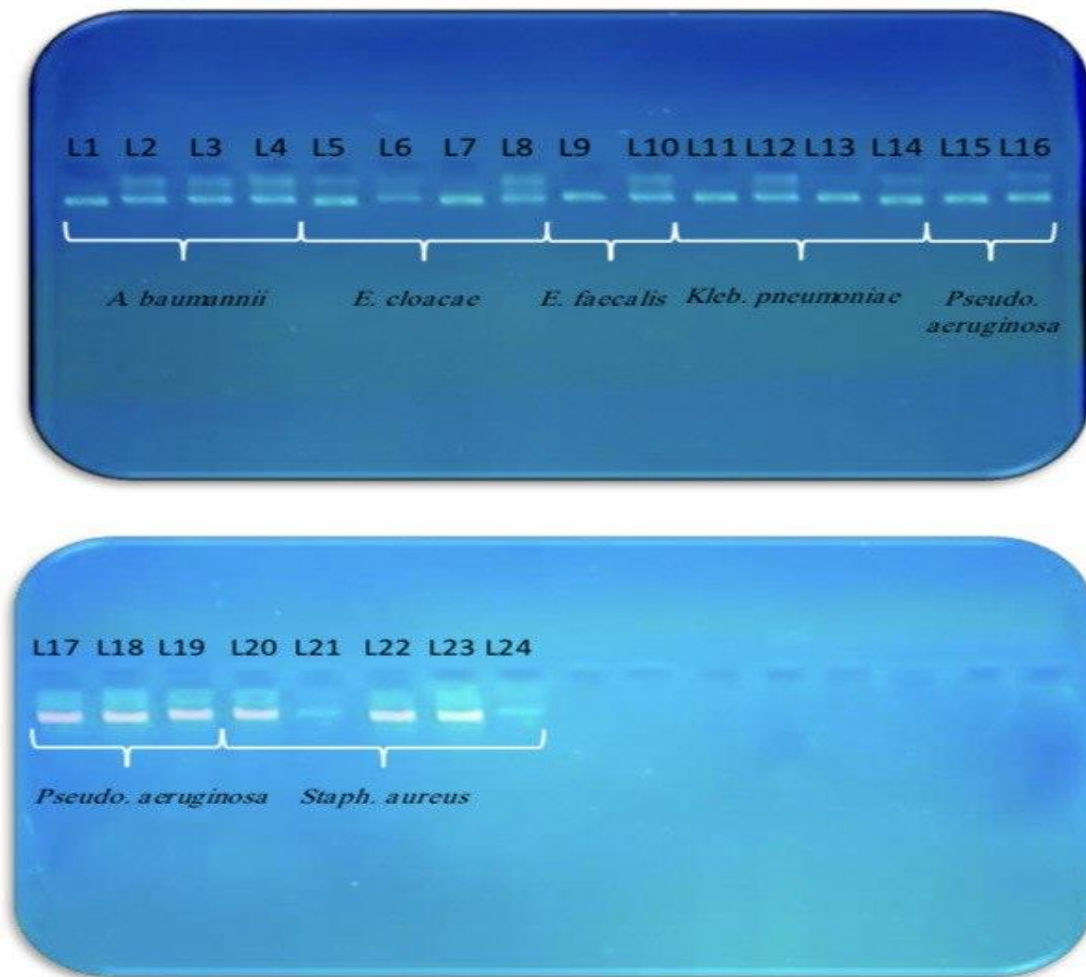


Fig. 1: Electrophoresis of Plasmid DNA isolated from bacterial strains on agarose gel.

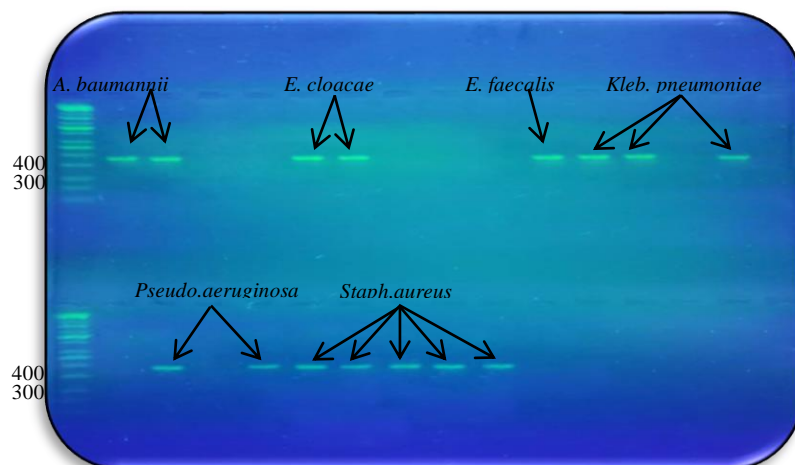


Fig-2: PCR amplification fragments for the detection of *acc(3)-II* gene(370bp) in bacterial Strains under study



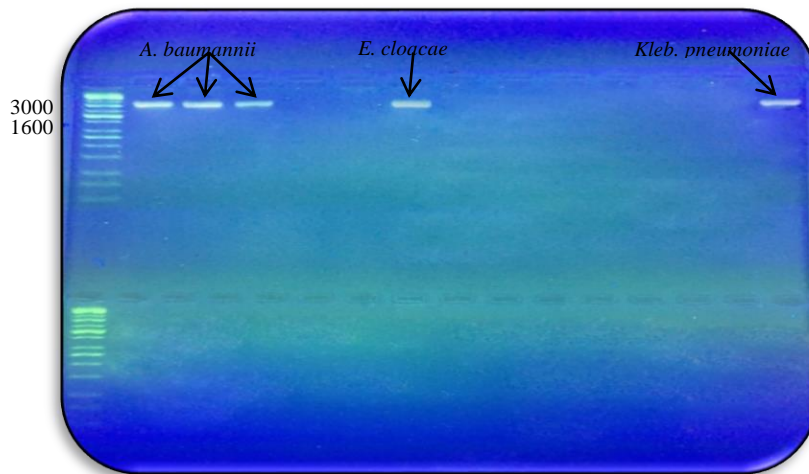


Figure 3. PCR amplification fragments for detecting *drg* gene(1980bp) in the bacterial strains under study.

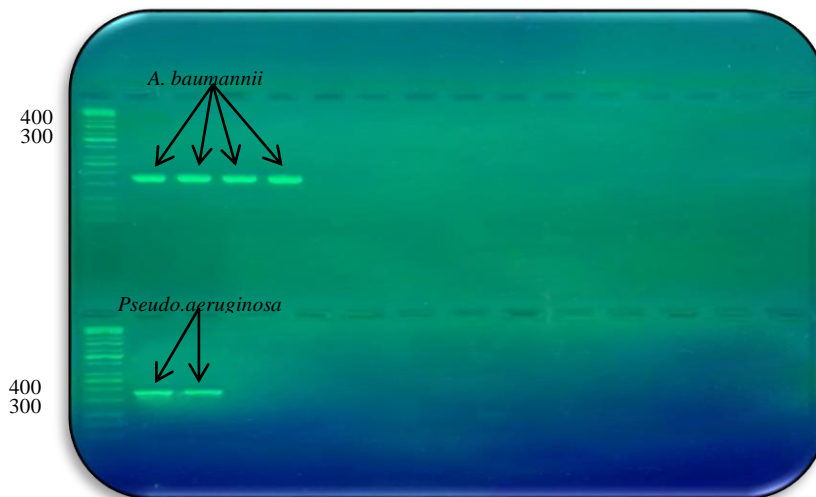


Figure 4. PCR amplification fragments for detecting *black-2* gene(369bp) in the bacterial strains studied.

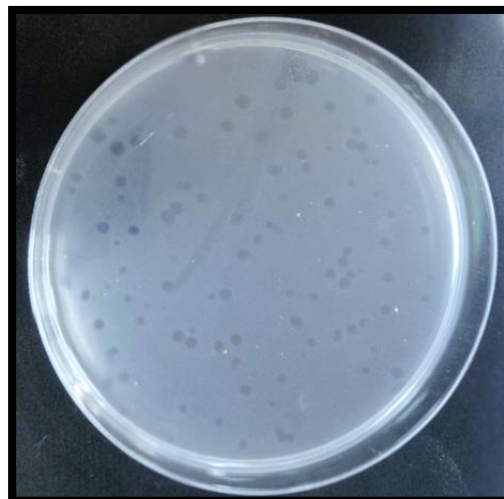


Figure 5: Plaque formation by bacteriophage.

## Discussion:

The consequences of the in vitro tradition propose that out of a total of 100 samples, 84 were examined for AB. The study conducted by [17] in Benin City, Nigeria indicated an occurrence of 45.3% among pregnant girls, whereas the prevalence of AB in nonpregnant women was 70.2%. In a recent have a look at carried out by [18], the researchers found a prevalence rate of 13.78% among pregnant individuals at Assosa General Hospital in western Ethiopia. In their study, [3] analyzed samples obtained from men, women, and individuals aged 70-80. The incidence ranged from 5% to 20% among aged males and from 20% to 50% among women. Furthermore, the research findings indicated a substantial prevalence of gramme-high-quality bacteria among both women and men, with costs of 45% and 14%, respectively. The effects of this look align with the research conducted by [19], which found a better occurrence of gram-effective isolates (sixty-three. 8% in the evaluation of Gramme-terrible bacteria (36.2%). [20] Based on the findings of the existing research, 67. Eight of the isolates acquired exhibited Gramme-tremendous traits, whereas 32.2% displayed Gramme-terrible traits. The researchers also mentioned *E. faecalis*, *S. aureus*, *E. coli*, and *Klebsiella pneumoniae* as the primary cardiomicrobial organisms. In a have a look performed by [21], it was shown that the bacteria maximum normally related to urinary tract infections (UTIs) were *E. fascia*, *E. Coli*, and *Staphylococcus aureus*. The effects of the existing research contradict the conclusions of a preceding Italian study conducted by [22], which stated that 50.4% of the bacterial traces collected were classified as Gramme-bad, even as 49.6% were categorized as Gramme-high-quality. Numerous examinations have validated the presence of Enterobacteriaceae germs in reproductive-age patients diagnosed with AB. According to the studies conducted by [23,24], it is viable that insufficient hygiene practices among ladies contribute to the transmission of gut plant life into

the genitourinary tract, resulting in contamination. In an examination performed by Krishnasamy *et al.* (2019), the prevalence of Gramme-negative microorganisms, especially *E. Coli*, *Pseudomonas spp.*, and *Klebsiella spp.*, was 15.2%, 6%, and 21.2%, respectively. [25] reported a similar outcome, indicating a prevalence fee of two for *A. baumannii* among infected girls. In the cutting-edge investigation, the incidence of *Enterococcus faecalis* was similar to that of *E. faecalis* (7.6%) in a previous nearby study [26].

The bacterium *Staphylococcus aureus* is responsible for each network-received and nosocomial infection within the human population. According to [27], there is evidence suggesting an upward push in methicillin-resistant *Staphylococcus aureus* infections in pregnant and postpartum women, as *Staphylococcus aureus* has the potential to colonize the lady's urinary tract and induce infections. *Staphylococcus aureus* is widely recognized for its potential to colonize mucous membranes and skin. Numerous studies have indicated that various traces of this bacterium can also colonize the urine of pregnant women, with a stated charge of as much as 22% [28]. The bacterium *Staphylococcus aureus* is responsible for each network-received and nosocomial infection within the human population. According to [27], there is evidence suggesting an upward thrust in methicillin-resistant *Staphylococcus aureus* infections in pregnant and postpartum women because of the colonization and next contamination as a result of *Staph. Aureus*. The colonization of mucous membranes, pores, and skin by *Staphylococcus aureus* is extensively regarded. Numerous studies have suggested the presence of numerous lines of this bacterium within urinary tract infections (UTIs) in pregnant women. The findings indicated that *Pseud.* Averages exhibited whole resistance to Piperacillin (a hundred%), at the same time as improved resistance to ceftazidime, tobramycin, gentamicin, piperacillin-tazobactam, aztreonam, Cefoperazone, and amikacin. They discovered that imipenem, Meropenem, and

gatifloxacin had low resistance charges, whereas ciprofloxacin, cefotaxime, and colistin had mild resistance. The study performed by [28], discovered that all isolates of *Pseud. Aeruginosa* exhibited complete resistance, with a resistance rate of 100%, to piperacillin.

In this investigation, the presence of the acc (three)-II gene was identified. The presence of the aforementioned strains, specifically *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Enterobacter cloacae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*, became discovered in a gram-terrible bacterium. Moreover, the presence of this gene was detected in a solitary high-quality bacterial isolate, particularly *Staphylococcus aureus*. [29] reported that the foremost AME gene changed into detected traces of *K. pneumoniae* and *Pseudomonas aeruginosa* acquired from patients admitted to hospitals, thereby corroborating the conclusions drawn in our study. Nevertheless, the gene in question was no longer identified by [30] in their research involving isolates obtained from patients at Najaf Hospital. In this investigation, drf was also detected in three wonderful species, along with *Klebsiella pneumoniae*, *Escherichia coli*, and *Acinetobacter baumannii*. This remark aligns with a study conducted by way of [31], in which they documented a comparable prevalence of dr in traces received from both terrestrial (thirteen.6 %) and aquatic (eleven.7 %) agricultural establishments. [32] recognized the presence of this gene in many bacterial strains, including *E. Coli* (33.1%) and *Klebsiella pneumoniae* (five. 3%, *Pseudomonas aeruginosa* (2%), and *Acinetobacter baumannii* (0.7%).

Additionally, the black-2 gene change into recognized in our investigation and was present exclusively in traces of *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. This commentary aligns with the findings of [33], which demonstrated the transferability of this gene between bacterial species including *Klebsiella pneumoniae* and

*Escherichia coli*. This gene was also detected in *E. Coli* and *Acinetobacter baumannii* was identified using [34], with incidence rates of 47.7% and 48.3%, respectively. Antibiotic susceptibility testing (AST GP) cards identified *Staphylococcus* spp. Shown a high degree of resistance (one hundred%) to penicillin and cephalosporin. In assessment, it was found that tigecycline exhibited the highest levels of sensitivity at 100, observed with the aid of daptomycin at 77.2%, and trimethoprim/sulfamethoxazole at 70 Four, vancomycin at 54. Five, and gentamicin at 52.2%. [35] mentioned comparable effects and revealed that *S. aureus* exhibited resistance fees of 67% and 33% for the antibiotics cited earlier. In most studies, *Staphylococcus* spp. Proven resistance quotes of seventy-two.7%, 63.6%, and forty-seven.7% towards tetracycline, Norfloxacin, and 47.7%, respectively.

Antibiotic susceptibility and resistance of *Enterococcus* spp. Assessed the use of many antibiotics, as provided in the findings presented in Table 6.

The bacteriophage titration of 20 plaques was determined to be 2 10<sup>2</sup> full. Phage remedy is an exceptionally promising area to investigate, regardless of the confined number of phages to be had for separated strains. [36] attempted to isolate bacteriophages specifically concentrated on Gramme-wonderful infections. A constraint of bacteriophage isolation culture media is that the phage should exhibit selectivity toward the tested bacteria and transparency to allow the detection of phage plaques.

## Conclusion

In conclusion, based on this study, we recommend routine screening for asymptomatic bacteriuria in all healthy males and females, conducted once every trimester through urine culture testing. This approach is crucial for preventing the risky complications associated with asymptomatic bacteriuria. The study findings indicate a prevalence

rate of 70.2% of asymptomatic bacteriuria in nonpregnant females and healthy males. Within this percentage, 61% were gram-positive and 23% were gram-negative.

#### Conflict of Interest:

All authors declare that they have no conflict of interest.

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