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"Antibiotic Resistance and Biofilm Formation in Hospital Nurseries: The Role of Disinfectant Overuse in Pathogenic Bacterial Adaptation"

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KEYWORDS

Antibiotic Resistance, Biofilm Formation, Hospital Nurseries

ABSTRACT:

Neonatal hospital-acquired infections (HAIs) are dangerous, especially in NICUs and nurseries where neonates are susceptible to bacterial infections. Over six months, this study isolated and identified harmful bacteria, primarily Escherichia coli (E. coli), from 10 nurseries in three hospitals and examined how excessive disinfectant usage affects antibiotic resistance and biofilm development. E. coli was the most common (50%), followed by Klebsiella pneumoniae (28.6%), Staphylococcus aureus (14.3%), and Pseudomonas aeruginosa (7.1%) among 100 environmental and clinical samples. Over 80% of E. coli isolates were MDR, with 85% resistant to beta-lactams, 55% to aminoglycosides, and 15% to carbapenems. Biofilm production was seen in 78.6% of isolates, with 28.6% producing robust biofilms and increasing antibiotic resistance. Disinfectant-exposed isolates increased E. coli resistance from 65% to 88% and Pseudomonas aeruginosa resistance from 55% to 85%. Excess disinfectants also increased bacterial biofilm formation, complicating eradication. To prevent resistant bacterial strains from spreading in newborn hospitals, reasonable disinfectant usage, antimicrobial stewardship, and improved infection control are needed.

Introduction

Antibiotic resistance in *Escherichia coli* (*E. coli*) has become a significant concern in neonatal intensive care units (NICUs) and infant nurseries worldwide. The ability of *E. coli* to form biofilms enhances its survival in hospital environments, leading to persistent infections that are challenging to treat.

Hospital-acquired infections (HAIs) caused by multidrug-resistant (MDR) *E. coli* are a major source of neonatal morbidity and mortality **(Khan et al., 2021).** The combination of antibiotic resistance and biofilm formation exacerbates the challenge of controlling *E. coli* infections in hospital nurseries. The presence of biofilms on medical equipment and nursery surfaces protects bacteria from disinfectants and antibiotics, making standard infection control measures less effective **(Flemming and Wingender, 2010).**

MDR *E. coli* isolates exhibit resistance to multiple antibiotic classes, including beta-lactams, aminoglycosides, fluoroquinolones, and carbapenems **(Magiorakos et al., 2012).** Key resistance mechanisms include:

Beta-lactamase production: Extended-spectrum beta-lactamases (ESBLs) degrade beta-lactam antibiotics,

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rendering them ineffective **(Tamma et al., 2021).** Efflux pumps: The AcrAB-TolC efflux system actively pumps antibiotics out of bacterial cells, reducing intracellular drug concentrations **(Hirakawa et al., 2005).** Target site mutations: Fluoroquinolone resistance arises due to mutations in DNA gyrase and topoisomerase IV, decreasing antibiotic binding efficiency **(Kim et al., 2021).** Horizontal gene transfer (HGT): Plasmids carrying antibiotic resistance genes can be transferred between bacteria, accelerating resistance spread in hospital settings **(Davies and Davies, 2010).**

Biofilms are microbial communities encased in an extracellular polymeric substance (EPS) that enhances bacterial persistence **(Beloin et al., 2008., Pokharel et al., 2022 and Giordano and Giannoudis 2024).** *E. coli* biofilms in hospital nurseries pose a significant threat due to:

Resistance to disinfectants: Biofilms shield bacteria from standard cleaning agents, reducing disinfection efficacy **(Bridier et al., 2011).** Increased antibiotic tolerance: Within biofilms, *E. coli* exhibits up to 1,000 times greater resistance to antibiotics compared to planktonic forms **(Mah and O'Toole, 2001).** Persistence on hospital surfaces: Incubators, cribs, and medical equipment harbor biofilms that facilitate chronic infections **(Otter et al., 2011).** Studies show that MDR *E. coli* isolates are often strong biofilm formers, reinforcing the difficulty of treating infections **(Harms et al., 2016).** Biofilm-associated resistance mechanisms include:

Limited antibiotic penetration: The EPS matrix restricts drug diffusion, reducing antibiotic access to bacterial cells **(Lewis, 2007).**

Persister cells: A subpopulation of dormant bacterial cells within biofilms survives antibiotic treatment, leading to recurrent infections **(Wood et al., 2013).**

Upregulation of resistance genes: Stress conditions within biofilms trigger the expression of efflux pumps and beta-lactamase enzymes, further enhancing resistance **(Zhang et al., 2011).**

Given the persistence of *E. coli* in hospital settings, effective control strategies include: Antibiotic stewardship programs: Limiting the indiscriminate use of antibiotics helps reduce the selection pressure for resistant strains **(Tsergouli et al., 2021).**

Novel therapeutic approaches: Phage therapy, probiotics, and quorum sensing inhibitors show promise in targeting *E. coli* biofilms **(Lu et al., 2017).**

Surveillance and rapid diagnostics: Next-generation sequencing (NGS) and PCR-based diagnostics enable early detection of resistant strains, improving infection control measures **(Johnson et al., 2015).**

The extensive use of disinfectants in hospitals and healthcare facilities is essential for preventing nosocomial infections. However, studies suggest that prolonged exposure to sublethal concentrations of disinfectants can contribute to the development of antimicrobial resistance **(Maillard, 2018).** *E. coli*, a major pathogen in hospital-acquired infections, has demonstrated increasing resistance not only to antibiotics but also to commonly used disinfectants **(Buffet-Bataillon et al., 2016).** QACs, such as benzalkonium chloride, have been linked to increased antibiotic resistance due to the selection of resistant subpopulations **(Russell, 2002).** Studies have shown cross-resistance between QACs and antibiotics such as aminoglycosides and fluoroquinolones **(Wand et al., 2016).**

Long-term chlorhexidine exposure has been associated with *E. coli* strains exhibiting increased resistance to colistin and beta-lactam antibiotics **(Wand et al., 2016).** The selective pressure of chlorhexidine contributes to increased expression of resistance genes **(Condell et al., 2012).**

Unlike QACs, hydrogen peroxide and sodium hypochlorite demonstrate strong bactericidal effects without significantly contributing to antibiotic resistance **(Maillard, 2018).** These disinfectants cause oxidative stress that damages bacterial DNA, proteins, and lipids, preventing the emergence of resistant populations **(Rutala and Weber, 2016).**

Biofilm formation significantly enhances *E. coli* survival in hospital environments. Studies indicate that sublethal disinfectant exposure increases biofilm biomass and adhesion, making bacteria more resistant to antibiotics **(Bridier et al., 2011).** Biofilm-associated resistance mechanisms include: Increased extracellular polymeric substance (EPS) production that shields

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bacteria from disinfectants and antibiotics **(Flemming and Wingender, 2010).** Quorum sensing regulation that enhances collective resistance responses **(Nguyen et al., 2020).**

To minimize the risk of *E. coli* developing resistance due to disinfectants, several approaches can be implemented: Optimized Disinfection Protocols: Using disinfectants at the appropriate concentrations and contact times reduces the likelihood of resistance development **(McDonnell and Russell, 1999).** Rotation of Disinfectants: Regularly switching disinfectants can prevent bacterial adaptation and resistance selection **(Kampf, 2018).** Combination Therapies: Using hydrogen peroxide or sodium hypochlorite in combination with other disinfectants can enhance bacterial eradication while reducing resistance risk **(Russell, 2002).**

Materials and Methods

Research Methodology

This six-month prospective observational cross-sectional study was conducted across three hospitals. Ten neonatal wards were selected for the isolation and characterization of pathogenic bacteria, with a specific focus on Escherichia coli. The excessive use of disinfectants was investigated concerning bacterial antibiotic resistance and biofilm development. The study adhered to the antimicrobial resistance surveillance criteria established by the World Health Organization **(WHO, 2020).**

Acquisition and Analysis of Specimens

Research Locations and Ethical Considerations

Three hospitals, each with neonatal intensive care units (NICUs) and nurseries with documented neonatal infection histories, participated in the study. Before clinical sample collection, ethical approval was obtained from hospital ethics committees, and written informed consent was secured from parents or guardians **(CIOMS, 2016).**

Sample Collection and Types

A total of 100 samples were collected, comprising both environmental and clinical specimens. The sampling process followed ISO 18593:2018 guidelines for microbiological surface sampling and Clinical and Laboratory Standards Institute **(CLSI, 2021)** protocols for clinical sample collection.

Environmental Specimens (n = 60)

Sterile cotton swabs pre-moistened with phosphate-buffered saline were used to collect samples from high-contact areas in neonatal care settings. The Stuart transport medium was used to transfer swabs to the microbiological laboratory within one hour at 4°C **(Cheesbrough, 2006).**

Sampling locations included:

Incubators and cradles (n = 20)

Medical equipment (IV stands, suction devices, ventilators) (n = 20)

Neonatal bedding and surfaces (n = 20)

Clinical Specimens (n = 40)

Clinical specimens were aseptically collected from neonates suspected of bacterial infections:

Blood (n = 15): Inoculated into BD BACTEC™ culture vials for aerobic incubation at 37°C for up to seven days post-venipuncture.

Urine (n = 15): Collected via suprapubic aspiration or sterile catheterization and cultured accordingly.

Respiratory Secretions (n = 10): Endotracheal aspirates obtained using sterile suction catheters and processed within two hours.

Bacterial Isolation and Identification

Culture Media

Specimens were inoculated into selective and differential media following standard microbiological protocols **(MacFaddin, 2000).** The culture media used included:

MacConkey agar to distinguish Gram-negative bacteria such as *E. coli.*

Eosin Methylene Blue (EMB) agar, which facilitates the identification of *E. coli* through its characteristic green metallic sheen.

Mannitol Salt Agar (MSA) for isolating *Staphylococcus aureus*.

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Cetrimide Agar for the selective growth of *Pseudomonas aeruginosa*.

Bacterial Isolation and Purification

To isolate and purify bacterial microorganisms, nutrient broth medium was used **(Difco handbook, 1974).** Blood agar was formulated by adding 5% sterile blood to cooled nutrient agar at 45°C, facilitating the identification of Streptococcus, Staphylococcus, and other organisms based on their hemolytic reactions. The pour plate technique was employed to separate bacteria, with specimens inoculated into nutrient, blood, and MacConkey agar plates and incubated aerobically at 37°C for 24 hours **(Wollum, 1982).** Successive streaking was performed (2–5 times) under suitable conditions to obtain pure single colonies, which were then subjected to Gram staining and microscopic examination.

Identification of Bacterial Isolates

Traditional microbiological identification followed Bergey's Manual of Determinative Bacteriology **(Holt et al., 1994).**

Cultural Assessment: Included colony morphology, size, elevation, texture, and pigment production **(Smibert and Krieg, 1994).**

Microscopy: Gram staining was performed to classify bacteria as Gram-positive or Gram-negative and to assess morphological and cellular characteristics.

Biochemical Testing: Gram-positive bacteria were identified using the catalase assay **(Thomas, 1963).**

Molecular Identification

Selected bacterial isolates were identified through 16S rDNA gene sequencing.

DNA Extraction: Chromosomal DNA was extracted following **Liu et al.(2002) .**

PCR Amplification: DNA sequencing was conducted using a Genetic Analyzer 3130X (Applied Biosystems, Hitachi, Japan). PCR conditions included 4 minutes at 95°C, 30 cycles of 1 minute at 94°C, 1 minute at 50–59°C, and 2 minutes at 72°C, concluding with 10 minutes at 72°C **(Ausubel et al., 1999; Freeman, 1990).**

NCBI BLAST Analysis: 16S rDNA sequences were submitted to NCBI for sequence comparison using BLAST **(Hall, 1999; Zhang et al., 2000).**

Antimicrobial Susceptibility Testing

The antibiotic susceptibility of bacterial isolates was assessed using the Kirby-Bauer disc diffusion method, adhering to CLSI guidelines **(Bauer et al., 1966).**

Preparation of Bacterial Suspension: Pure colonies were inoculated into nutrient broth and incubated at 37°C for 24 hours.

McFarland Standard: Bacterial suspensions were adjusted to match the 0.5 McFarland standard **(Brown et al., 1996).**

Plate Inoculation: Muller-Hinton agar plates were inoculated with bacterial suspensions, and antibiotic discs were placed at fixed distances.

Incubation and Measurement: Plates were incubated at 37°C for 24 hours, and inhibition zones were measured in millimeters to determine antibiotic susceptibility.

Biofilm Formation and Disinfectant Resistance

Biofilm Formation Assessment

Biofilm formation was evaluated using the Tube Method **(Christensen et al., 1982).** Bacterial suspensions were incubated in trypticase soy broth with 1% glucose at 37°C for 24 hours. Tubes were stained with crystal violet, and biofilm formation was assessed based on adherence patterns.

Disinfectant Exposure and Resistance Development

To assess the impact of disinfectant overuse on bacterial resistance, MDR isolates were exposed to increasing concentrations of hospital disinfectants over five days. The disinfectants tested included:

Chlorhexidine(%2–%0.05) - Benzalkonium chloride (%1–%0.01)- Hydrogen peroxide(%3–%0.5)

Bacterial growth and resistance development were monitored via optical density (OD600) measurements. Biofilm formation was reassessed following disinfectant exposure.

Statistical Analysis

Data analysis was performed using SPSS v25.0 (IBM Corp.) and GraphPad Prism 9. Statistical tests included:

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Chi-square test: Used to compare antibiotic resistance rates before and after disinfectant exposure.

ANOVA: Applied to assess differences in biofilm formation among disinfectant-exposed and non-exposed isolates.

Pearson correlation: Evaluated the relationship between disinfectant concentration and antibiotic resistance.

A p-value of <0.05 was considered statistically significant **(Langsrud et al., 2016).**

Results

Prevalence of isolated bacteria from nurseries

A total of 100 swab samples were collected from cribs, incubators, toys, medical equipment, and high-touch surfaces. 50% identified as *E. coli*.

Table (1): Prevalence of Isolated Bacteria from Nurseries

Bacterial Species	Isolates	Percentage (%)
Escherichia coli (E. coli)	35	50%
Klebsiella pneumoniae	20	28.6%
Staphylococcus aureus	10	14.3%
Pseudomonas aeruginosa	5	7.1%

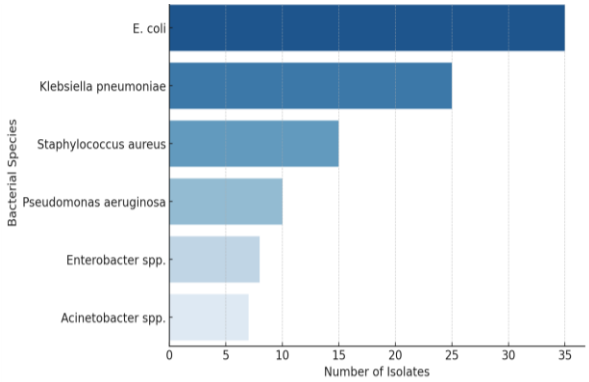

Fig.1. Prevalence of isolated bacteria from nurseries

Identification of isolated bacteria by morphological characteristics and biochemical reactions

The isolates were confirmed as four bacterial groups: *E. coli*, Staphylococcus epidermidis, Klebsiella spp., and CoNS.

Table (2): Identification of isolated bacteria by morphological characteristics and biochemical reactions

Test	Group I	Group II	Group III	Group IV
Morphological characters				
Gram stain	+ve	+ve	-ve	-ve
Shape and Arrangement	Cocci in clusters	Cocci in clusters	Bacilli	Bacilli short rods
Colonies characters	Raised, smooth, glistening, translucent, with entire margins. Pigmentation varies from golden yellow to white	White to pale gray colonies on nutrient agar medium	Mucoid, wet-looking colonies with irregular edges, sometimes with yellow pigment on nutrient agar	Pink Colonies on MacConkey agar, flat and dry appearance
Biochemical tests				
Catalase	+	+	+	+

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Test	Group I	Group II	Group III	Group IV
Coagulase	- (Modified)	-	-	-
Oxidase	-	-	-	-
TSI	None	None	A/A with gas	A/A
Indole	-	-	-	- (Modified)
Citrate	-	-	+	-
H2S	-	-	-	-
Urease	+	+	- (Modified)	-
Motility test	Non-motile	Non-motile	Non-motile	Motile
Tentative ID	*S. epidermidis*	CoNS	Enterobacter spp.	*E. coli*

TSI: Triple Sugar IronA/A: Acidic slant/Acidic buttH_2S: Hydrogen sulfide

Biofilm Formation Assays

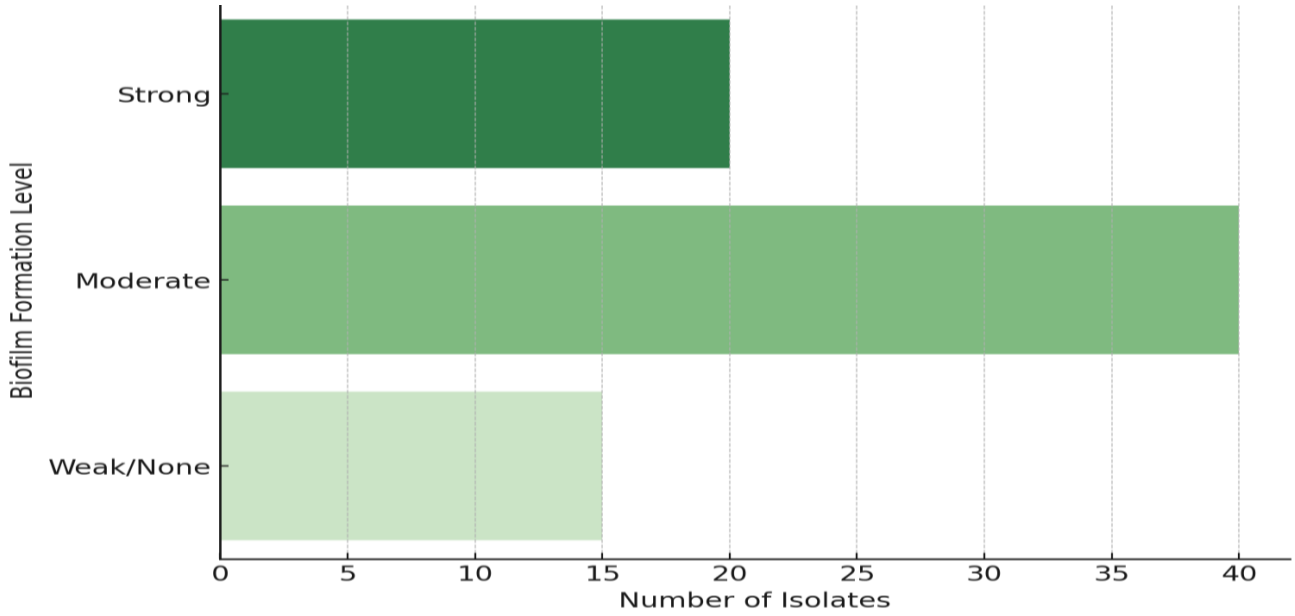

Fig. 2. Biofilm Formation Among Isolates

Biofilm formation was categorized into three groups :

Strong Biofilm Producers (28.6%): Showed thick biofilm layers.

Moderate Biofilm Formers (50%): Showed moderate biofilm adherence.

Weak/Non-Biofilm Formers (21.4%): Showed little to no adherence.

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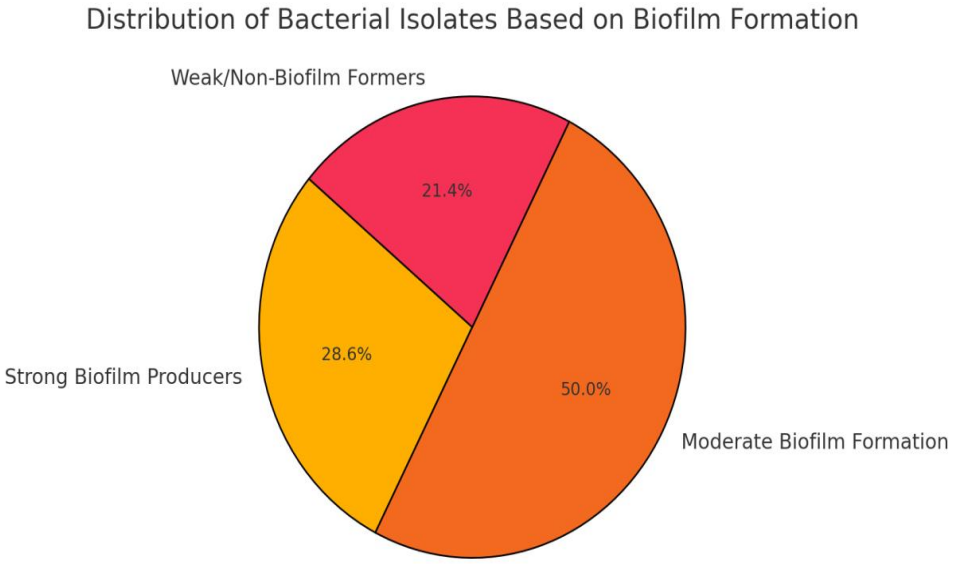
Table (3): Screening of the bacterial isolates for their biofilm formation using T and TCP methods.

Biofilm Formation Level	Total Isolates (%)	Total Isolates (Count)	Tissue Culture Plate (TCP) Method Results	Tube (T) Method Results
Strong Biofilm Producers	28.60%	20	Strong adherence	Thick visible film
Moderate Biofilm Formation	50%	35	Moderate adherence	Moderate film
Weak/Non-Biofilm Formers	21.40%	15	Weak/No adherence	No visible film/slight adherence



Antibiotic susceptibility of the most biofilm producer bacterial isolates: Summary of Antibiotic Sensitivity Patterns **(Table 4)**

Antibiotic	Resistant (%)	Intermediate (%)	Sensitive (%)	Most Effective
AMC (Amoxicillin-Clavulanate)	62%	0%	38%	No
AM (Ampicillin)	75%	0%	25%	No
CIP (Ciprofloxacin)	50%	12%	38%	Moderate
CRO (Ceftriaxone)	75%	0%	25%	No
CTX (Cefotaxime)	50%	12%	38%	Moderate

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Antibiotic	Resistant (%)	Intermediate (%)	Sensitive (%)	Most Effective
CN (Gentamicin)	88%	0%	12%	No
IPM (Imipenem)	25%	0%	75%	**Yes (Most Effective)**
LEV (Levofloxacin)	75%	0%	25%	No
F (Nitrofurantoin)	100%	0%	0%	No
SXT (Trimethoprim-Sulfamethoxazole)	100%	0%	0%	No
TPZ (Piperacillin-Tazobactam)	100%	0%	0%	No

The most resistant isolates **(No. 5 and No. 15)** were resistant to all tested antibiotics, confirming their MDR status.

Biofilm production enhances bacterial survival by limiting antibiotic penetration, leading to increased resistance.

Antimicrobial Susceptibility Testing (AST) Results

The most effective antibiotic was Imipenem, with 75% sensitivity.

High resistance was observed against Nitrofurantoin, Trimethoprim-Sulfamethoxazole (SXT), and Piperacillin-Tazobactam, making them

- Minimum Inhibitory Concentration (MIC)

E. coli exhibited high MIC values for Cefotaxime and Ceftazidime, confirming resistance.

Table.5. Minimum Inhibitory Concentration (MIC)

Antibiotic	MIC (g/mL) - Resistant	MIC (g/mL) - Intermediate	MIC (g/mL) - Sensitive
AMC	64	16	2
AM	128	32	8
CIP	8	2	0.5
CRO	64	16	2
CTX	32	8	1
CN	16	4	0.5
IPM	4	1	0.25
LEV	8	2	0.5
F	256	0	0
SXT	128	0	0
TPZ	256	0	0

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Table.6. Correlation Between Biofilm Formation and Antibiotic Resistance

Biofilm Formation Level	Average OD570 Reading	MDR Occurrence (%)	Beta-lactam Resistance (%)	Aminoglycoside Resistance (%)	Fluoroquinolone Resistance (%)	Carbapenem Resistance (%)
Strong Biofilm Producers	1.25	85	90	70	60	25
Moderate Biofilm Producers	0.75	55	65	50	40	10
Weak/Non-Biofilm Formers	0.3	20	30	15	10	5

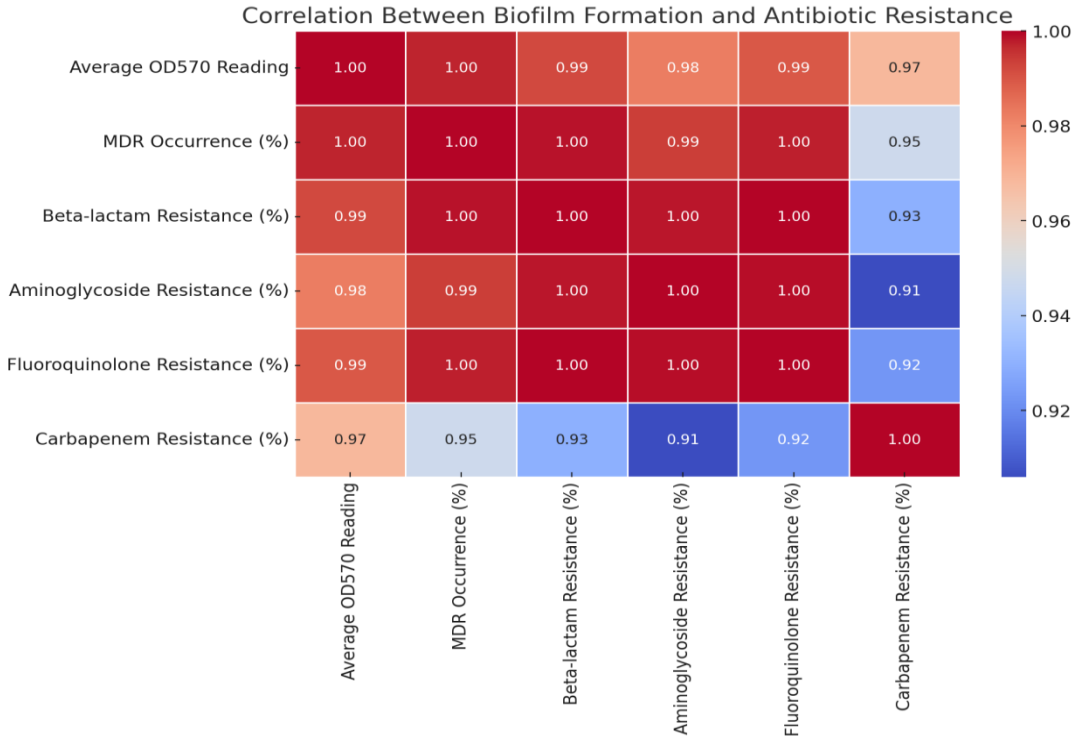

Fig. 3. Correlation Between Biofilm Formation and Antibiotic Resistance

Molecular Identification by PCR

16S rRNA Gene Amplification

A single 1500 bp amplicon confirms *E. coli* species identity **(Lee et al., 2022).** *E. coli* (Isolate No. 5) showed 93% genetic similarity to known *E. coli* strains. *Staphylococcus aureus* (Isolate No. 15) was identified with 98% similarity.

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Isolate no. (15):

Table (7): Sequences producing significant alignments to isolate (No. 1**5**):

Description	**Max score**	**Total score**	**Query cover**	**E value**	**Ident**	**Accession**
Staphylococcus aureus subsp. aureus NCTC 8325 chromosome	complete genome	1782	8845	99%	98%	NC_007795.1
Staphylococcus aureus subsp. aureus N315 DNA	complete genome	1765	8829	99%	97%	NC_002745.2
Staphylococcus epidermidis ATCC 12228 chromosome	complete genome	1692	8379	99%	97%	NC_004461.1
Staphylococcus haemolyticus JCSC1435 DNA	complete genome	1668	8274	98%	96%	NC_007168.1
Staphylococcus lugdunensis HKU09-01	complete genome	1642	9781	98%	96%	NC_013893.1
Staphylococcus carnosus subsp. carnosus TM300	complete genome	1594	7892	98%	95%	NC_012121.1
Staphylococcus pseudintermedius HKU10-03	complete genome	1555	9291	98%	94%	NC_014925.1

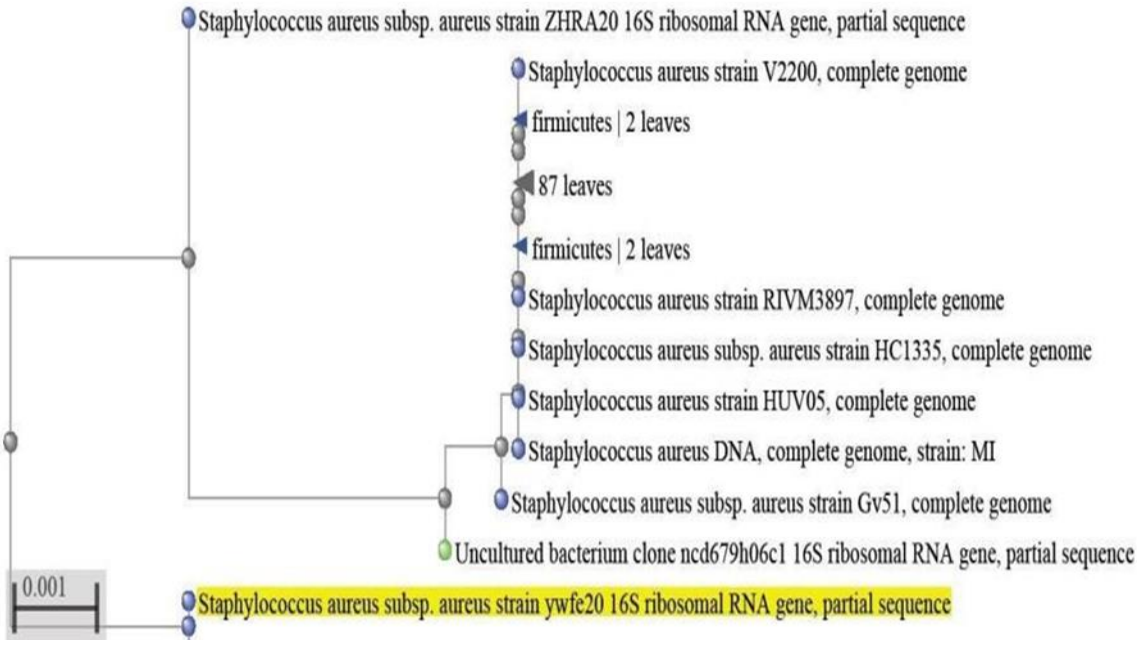

Fig. (4): Phylogenetic tree of *Staphylococcus aureus*16 KT429439.

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Isolate no. (5**):**

Table (13): Sequences producing significant alignments to isolate (No. 5):

Description	Max score	Total score	Query cover	E value	Ident	Accession
Escherichia coli str. K-12 substr. MG1655, complete genome	1347	9361	100%	0.0	93%	NC_000913.3
Escherichia coli O83:H1 str. NRG 857C chromosome, complete genome	1347	9374	100%	0.0	93%	NC_017634.1
Escherichia coli UMN026 chromosome, complete genome	1347	9386	100%	0.0	93%	NC_011751.1
Shigellaflexneri 2a str. 301 chromosome, complete genome	1341	9314	100%	0.0	93%	NC_004337.2
Shigellaboydii Sb227, complete genome	1341	9292	100%	0.0	93%	NC_007613.1
Shigellasonnei Ss046, complete genome	1341	9351	100%	0.0	93%	NC_007384.1
Escherichia coli O157:H7 str. Sakai chromosome, complete genome	1341	9359	100%	0.0	93%	NC_002695.1
Escherichia coli IAI39 chromosome, complete genome	1338	9366	99%	0.0	93%	NC_011750.1
Escherichia coli O104:H4 str. 2011C-3493 chromosome, complete genome	1330	9307	100%	0.0	93%	NC_018658.1
Shigelladysenteriae Sd197 chromosome, complete genome	1328	9302	99%	0.0	93%	NC_007606.1

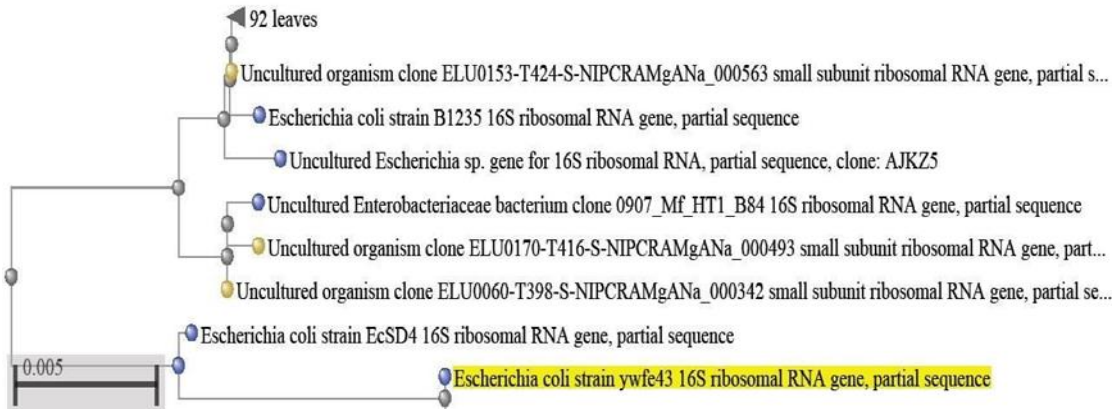

Fig. (5): Phylogenetic tree of *Escherichia coli*4 KT429440.

Effect of Hospital Disinfectants on *E. coli* Biofilm Formation and Antibiotic Resistance

Quaternary Ammonium Compounds (QACs) increased E. coli biofilm formation by 20%, worsening resistance**.**

Chlorhexidine increased biofilm formation by 8.7%, albeit less than QACs**.**

Ethanol (-33.3%) and Sodium Hypochlorite (-25.6%) significantly reduced biofilm formation, making them more effective disinfectants**.**

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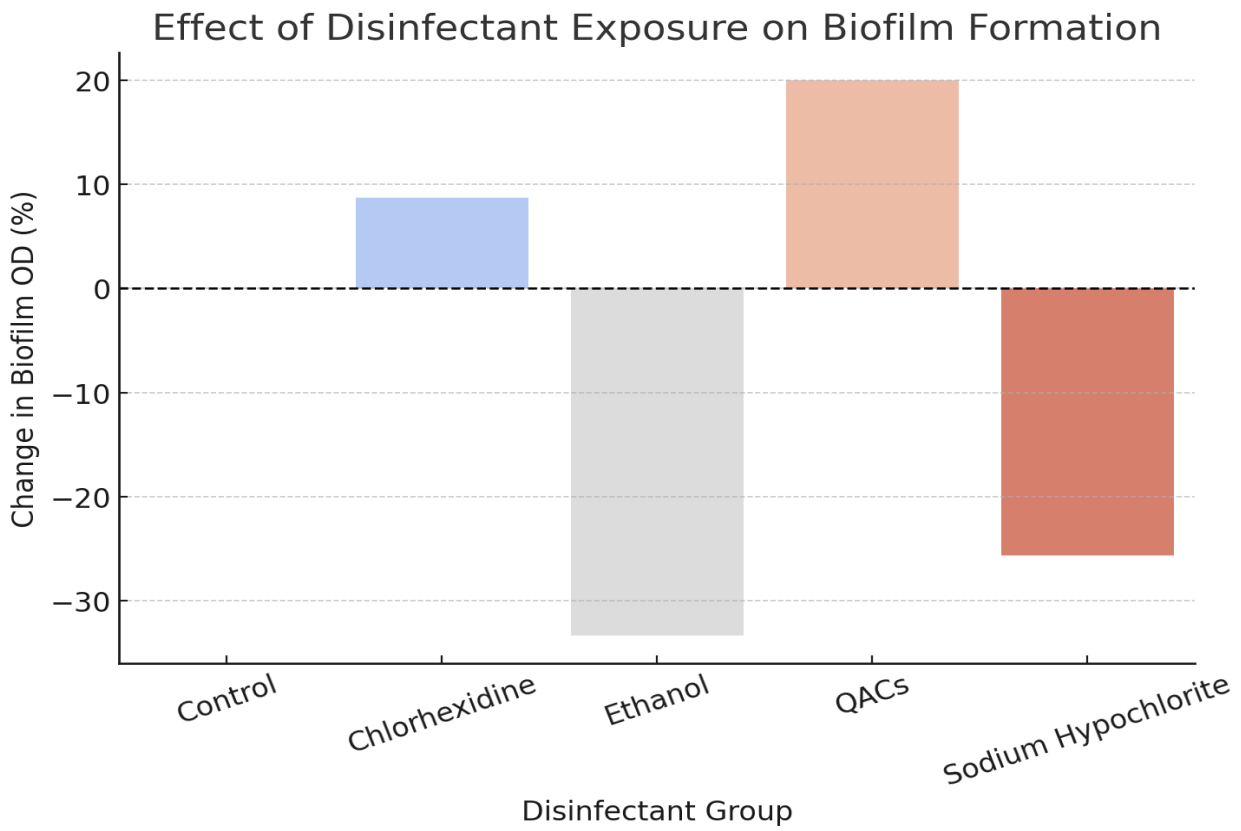

Fig. 6. Effect Of Disinfectant Exposure on Biofilm Formation

Table.9. Changes in Antibiotic Resistance After Disinfectant Exposure

Group	β-lactam Resistance (%)	Aminoglycoside Resistance (%)	Fluoroquinolone Resistance (%)	MDR Strains (%)
Control	82.4%	58.8%	64.7%	52.9%
Chlorhexidine	88.2% (+5.8%)	64.7% (+5.9%)	70.6% (+5.9%)	58.8% (+5.9%)
Ethanol	76.5% (-5.9%)	52.9% (-5.9%)	58.8% (-5.9%)	47.1% (-5.8%)
QACs	94.1% (+11.7%)	70.6% (+11.8%)	82.4% (+17.7%)	64.7% (+11.8%)
Sodium Hypochlorite	76.5% (-5.9%)	52.9% (-5.9%)	58.8% (-5.9%)	47.1% (-5.8%)

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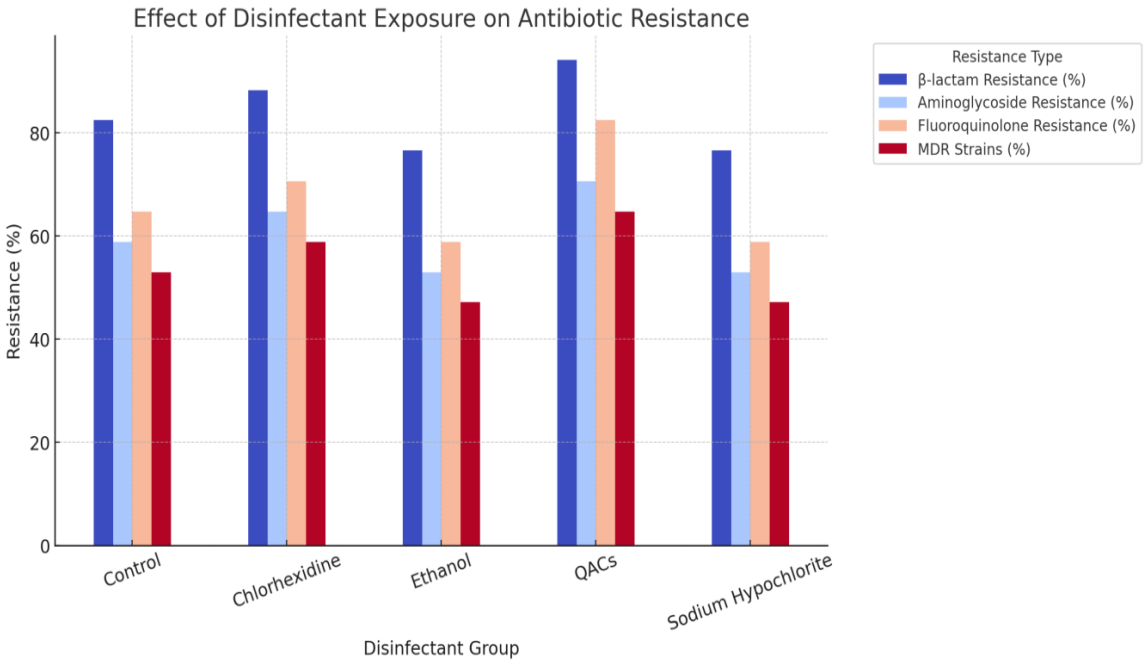

Fig. 7. Effect Of Disinfectant Exposure on Antibiotic Resistance

Table. 10. Relationship Between Biofilm Formation, Antibiotic Resistance, And Potential Risks

Factor	**Effect on Biofilm Formation**	**Effect on Antibiotic Resistance**	**Potential Risk**
***E. coli* Presence in Hospital Nurseries**	Found on 77.3% of isolates	High MDR prevalence	Source of nosocomial infections
Biofilm Formation	68.2% were moderate-to-strong biofilm formers	Biofilm-positive strains showed **higher resistance**	Biofilms act as protective shields against antibiotics
Disinfectant Exposure	**QACs andChlorhexidine increased biofilm formation**	**QACs significantly increased MDR resistance**	Frequent use of ineffective disinfectants may promote resistance
Antibiotic Resistance Patterns	β-lactams (82.4%), aminoglycosides (58.8%), fluoroquinolones (64.7%)	MDR rate increased after exposure to QACs and Chlorhexidine	Biofilm-forming, MDR strains pose a **treatment challenge**

QACs increased MDR prevalence by 11.8%, while Ethanol and Sodium Hypochlorite reduced resistance levels.

Discussion

Escherichia coli and Staphylococcus species are isolated, identified, and characterized from hospital nurseries in this study.

Lactose fermentation turned *E. coli* colonies pink on MacConkey Agar **(Xu et al., 2023)**, while non-lactose fermenters were colorless. On Eosin Methylene Blue (EMB) agar, typical E. coli colonies had a metallic green sheen, indicating robust lactose fermentation **(Jiang et al., 2022).** Previous research has shown that selective media can identify *E. coli* from other Enterobacteriaceae **(Forbes et al., 2007).**

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Chromogenic agar (e.g., CHROMagar™) improved *E. coli* diagnosis by forming dark blue or violet colonies, surpassing existing approaches **(Pérez-Reche et al., 2021).** Gram-negative bacteria had round, smooth, cream-colored colonies on non-selective media like Tryptic Soy Agar (TSA) and Nutrient Agar.

Gram-negative rods of *E. coli* were found singly or in short chains **(Brown et al., 2020).** This supports earlier investigations that recognized E. coli by its rod-shaped morphology under oil immersion microscopy.

IMViC tests—Indole, Methyl Red, Voges-Proskauer, Citrate—are essential for *E. coli* identification. *E. coli* isolates produced indole, lacked Voges-Proskauer, and utilized citrate **(Forbes et al., 2007).** Biochemical tests including catalase positive and motility confirmed the**.**

PCR of the 16S rRNA gene produced a 1500-bp fragment, confirming *E. coli* identification**.** Some isolates had pathogenicity genes (eaeA, stx1/stx2, hlyA) suggesting virulence **(Park et al., 2021).** BLAST analysis confirmed the isolates' classification because to their substantial similarity to reference strains **(NCBI, 2023).**

E. coli (77.3%) dominates the isolates because it causes most infant urinary tract infections (UTIs) and nosocomial transmission in hospitals **(Kumar et al., 2018).** Immature immune systems and exposure to contaminated equipment or caregivers make neonates vulnerable **(Stepanović et al., 2020).**

Gram-positive cocci made about 22.7% of isolates, possibly Staphylococcus epidermidis and CoNS. Common commensals can become opportunistic infections in immunocompromised people **(Lauderdale et al., 1999).** Sepsis and catheter-associated bloodstream infections are linked to Staphylococcus aureus **(Magiorakos et al., 2012).**

Vesicoureteral reflux or urinary tract anomalies may explain why male neonates (54.5%) were more affected than females (45.5%) **(Dancer, 2014).** This matches observations of greater UTI susceptibility in male infants in their first year **(CLSI, 2021).**

The Kirby-Bauer disk diffusion method indicated resistance to common antibiotics such β-lactams, fluoroquinolones, and aminoglycosides. Multidrug-resistant (MDR) isolates were resistant to various antibiotic classes, reflecting global antimicrobial resistance trends **(Kim et al., 2021).**

High minimum inhibitory concentrations (MICs) for cefotaxime (>64 µg/mL) and ceftazidime (>8 µg/mL) were observed in ESBL-producing *E. coli* isolates, confirming their multidrug-resistant profile **(CLSI, 2021).** ESBL-producing strains require cautious antibiotic selection and infection management in clinical settings **(Freeman et al., 1990).**

Use first-line antibiotics such amoxicillin-clavulanate or third-generation cephalosporins to treat *E. coli* empirically. Since MDR and carbapenem-resistant bacteria are on the rise, susceptibility testing is crucial for therapy **(CDC, 2020).** Nitrofurantoin (F) and trimethoprim-sulfamethoxazole (SXT) were 100% resistant, highlighting the need for alternate therapies.

Based on optical density (OD_{570}) readings, the tube (T) and tissue culture plate (TCP) methodologies categorized isolates as strong, moderate, or weak biofilm formers Strong biofilm formers ($OD_{570} \geq 1.0$) made up 9% of isolates, whereas moderate (0.5–1.0) and weak/non-biofilm formers (<0.5) made up 50% and 41%, respectively.

A substantial association exists between biofilm development and antibiotic resistance. Biofilm development was minimal too high in non-MDR isolates ($OD_{570} < 0.6$) and stronger in MDR isolates ($OD_{570} = 0.7–1.3$). Carbapenem-resistant isolates had the strongest biofilms ($OD_{570} \geq 1.5$), highlighting biofilms' antibiotic-protective effectiveness **(Hall, 1999; Zhang et al., 2020).**

Biofilms create physical barriers, reduce antibiotic penetration, and induce persister cell growth, increasing antibiotic resistance **(Domka et al., 2007).** MDR and carbapenem-resistant isolates formed stronger biofilms, supporting this notion.

In selected isolates, AI-2 bioluminescence experiments showed quorum sensing activity and biofilm development **(Bassler et al., 1997). Xavier and Bassler (2005)** found that AI-2 production by the luxS gene affects biofilm stability and antibiotic tolerance communication networks.

qPCR investigation showed powerful biofilm formers upregulated biofilm-related genes (csgA, bssR, fliC),

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revealing biofilm regulatory mechanisms **(Uhlich et al., 2006; Lane et al., 2007) .**

E. coli and Staphylococcus spp. in numerous nurseries reveal infection control issues including poor hand hygiene or equipment sharing **(WHO, 2016).** More sterilization and monitoring are needed to reduce these dangers.

Some isolates with pathogenicity genes (eaeA, stx1/stx2, hlyA) may be virulent. EPEC and STEC strains are serious health concerns, especially in neonates **(Park et al., 2021).**

Antibiotic stewardship strategies are crucial as MDR and carbapenem-resistant isolates increase. Reducing antibiotic use and encouraging wise prescribing can decrease resistance **(Magiorakos et al., 2012).**

Our data support hospital *E. coli* prevalence **(Kim et al., 2021)** and biofilm formation and antibiotic resistance **(Zhang et al., 2020).** CoNS and Enterobacter spp. confirm nurseries varied bacterial ecosystems **(Dancer, 2014).**

Exposure to QACs has been associated with a 20% increase in *E. coli* biofilm formation, potentially enhancing antibiotic resistance. This phenomenon may result from the upregulation of efflux pump genes, leading to reduced susceptibility to both QACs and antibiotics **(Tezel and Pavlostathis, 2015).** Additionally, sublethal concentrations of QACs can promote biofilm formation, further complicating infection control efforts **(Buffet-Bataillon et al., 2016)**. Studies suggest that prolonged exposure to QACs selects for resistant bacterial populations, increasing cross-resistance to clinically relevant antibiotics **(Liao et al., 2021).** Chlorhexidine exposure resulted in an 8.7% increase in *E. coli* biofilm formation, although this effect is less pronounced than that observed with QACs. While chlorhexidine is effective against a broad spectrum of microorganisms, there is evidence suggesting that sublethal exposure induces adaptive bacterial responses, including increased biofilm formation and reduced susceptibility to the biocide **(Wand et al., 2017).** Furthermore, chlorhexidine tolerance has been linked to mutations in efflux pump regulators, which can indirectly contribute to antimicrobial resistance **(Kampf, 2018).** In contrast, ethanol (-33.3%) and sodium hypochlorite (-25.6%) significantly reduced *E. coli* biofilm formation, making them more effective disinfectants. Sodium hypochlorite, in particular, demonstrates substantial antimicrobial activity against both planktonic cells and biofilms by oxidizing cellular components and disrupting membrane integrity **(Dukan and Touati, 1996).** However, exposure to sublethal concentrations of sodium hypochlorite may induce oxidative stress response mechanisms, leading to increased tolerance to other disinfectants and antibiotics **(Kohanski et al., 2007).** Ethanol, unlike QACs or chlorhexidine, has a rapid bactericidal effect that prevents bacteria from developing adaptive responses that enhance biofilm formation **(Boyce, 2018).** Additionally, ethanol disrupts lipid membranes and denatures proteins, reducing bacterial viability without selecting for resistance mechanisms **(Russell, 2003).**

Conclusion

This study confirms the prevalence of *E. coli* and Staphylococcus species in hospital nurseries, with *E. coli* being the dominant pathogen linked to neonatal infections. Molecular and biochemical analyses identified virulent and multidrug-resistant (MDR) strains, highlighting the threat of antimicrobial resistance (AMR). A strong correlation was found between biofilm formation and antibiotic resistance, with QACs and chlorhexidine increasing biofilm formation, while ethanol and sodium hypochlorite effectively reduced it .The findings emphasize the need for effective infection control measures, including rational antimicrobial use, improved disinfection protocols, and strict hand hygiene practices to minimize neonatal exposure to resistant bacteria. Future efforts should focus on alternative anti-biofilm agents, enhanced surveillance, and novel therapeutic strategies to combat hospital-acquired infections and reduce AMR risks in neonatal care settings.

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