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Clinical and Molecular Characterization of Viral-Bacterial Co-Infections in Upper Respiratory Tract Infections: Pathogen Profiles and Antimicrobial Resistance Patterns

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ABSTRACT:

Background: Upper respiratory tract infections are among the prevalent infections at the present time, owing to the multiple viral and bacterial pathogens targeting the upper respiratory system. These infections can only be detected through the use of advanced diagnostic techniques, such as RT-PCR systems, which enable the identification of the most prominent viruses involved in upper respiratory tract infections, including Coronavirus, Influenza, Parainfluenza, RSV, and Adenovirus. . Bacterial isolates (Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Moraxella catarrhalis) were identified via standard microbiological procedures and API biochemical systems. Antimicrobial susceptibility testing (AST) was performed using the Kirby-Bauer disk diffusion method according to CLSI guidelines.

Results: the 500 samples, single viral infections were detected in 38.4% (n=192), single bacterial infections in 22.0% (n=110), and viral-bacterial co-infections in 28.4% (n=142). The most frequent co-infection profile involved Influenza A combined with Streptococcus pneumoniae (31.0%), followed by RSV with Staphylococcus aureus (22.5%). Patients with co-infections exhibited significantly higher clinical severity scores compared to those with viral mono-infections ($p < 0.001$). Furthermore, bacterial strains isolated from co-infected cases demonstrated a strikingly higher rate of multidrug resistance (MDR) compared to mono-bacterial isolates (64.8% vs. 41.8%, $p = 0.002$

1. INTRODUCTION

Upper respiratory tract infections (URTIs) are among the most common infectious diseases treated in clinical settings worldwide [1]. While the primary etiology is overwhelmingly viral—predominantly driven by Influenza viruses, Respiratory Syncytial Virus (RSV), Adenovirus, and Rhinoviruses—secondary bacterial superinfections frequently complicate the clinical course [2].The pathogenic mechanism underlying viral-bacterial synergy is complex. إليك ترجمة علمية دقيقة ومحكمة للنص باللغة الإنجليزية، بصياغة أكاديمية رصينة مناسبة لمقدمة أو منهجية أوراق البحث العلمي:

> "Viral-bacterial synergy leads to a complex pathogenic mechanism. Initial viral replication causes damage to the mucosal membranes and disrupts tight junctions between epithelial cells. This impairs mucociliary clearance and dysregulates the host's innate immune response, thereby facilitating secondary bacterial colonization and infection by various bacterial pathogens. Furthermore, conventional outpatient diagnostic algorithms predominantly focus on identifying a single isolated pathogen, while ignoring the complex co-infection dynamics occurring between viruses and bacteria. Consequently, this contributes to

rising rates of antimicrobial resistance. Therefore, this study aims to investigate the prevalence, viral-bacterial co-occurrence patterns, and antimicrobial resistance profiles in upper respiratory tract infection samples using RT-PCR techniques."

2. MATERIALS AND METHODS

2.1 Sample Collection and Processing

A total of 500 clinical samples were collected from outpatient clinics and hospitals, comprising patients presenting with upper respiratory tract infections. Nasopharyngeal/oropharyngeal swabs were obtained using sterile swabs and immediately immersed in Viral Transport Medium (VTM). The collected samples were transported under refrigerated conditions (4°C) and subsequently stored at -20°C for downstream laboratory assessments.

2.2 Molecular Detection of Viral Pathogens

Total viral RNA/DNA was extracted using a commercial viral nucleic acid extraction kit according to the manufacturer's protocol. Multiplex real-time RT-PCR assays were performed to detect key respiratory viruses, including:

- Influenza A and B
- Respiratory Syncytial Virus (RSV)
- Adenovirus (AdV)
- Human Parainfluenza Viruses (HPIVs)

Amplification was carried out on a real-time PCR thermal cycler, with target-specific primers and fluorescent probes.

Bacterial Isolation and Identification:

Following transport to the laboratory, samples were inoculated onto Blood Agar, Chocolate Agar, and MacConkey Agar plates. The cultures were incubated aerobically at 37°C for 24 hours. Bacterial isolates were initially characterized by colony morphology and microscopic examination using Gram staining. Subsequently, standard biochemical tests and API test strips were performed to confirm the identification of the isolated bacterial species.

2.4 Antimicrobial Susceptibility Testing (AST)

Antimicrobial susceptibility profiles were determined using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar. Inactivation zone diameters were interpreted in accordance with the latest Clinical and Laboratory Standards Institute guidelines (CLSI, 2025). Strains resistant to at least one agent in three or more antimicrobial categories were classified as Multidrug-Resistant (MDR).

2.5 Statistical Analysis

Data analysis was conducted using Jamovi (version 2.6.44) Categorical variables were expressed as frequencies and percentages and comared using Pearson's Chi-square (chi^2) test or Fisher's exact test. Independent samples t-tests were utilized for continuous variables. A $p-$value of < 0.05 was considered statistically significant

3. RESULTS

Bacterial Isolation & Identification

Clinical Bacterial Isolation and Cultivation

Bacterial pathogens were clinically isolated from patients diagnosed with upper respiratory tract infections (URTIs). The collected clinical specimens were cultured on standard bacteriological culture media. Following incubation, microscopic, morphological, and biochemical evaluations confirmed the identification of several Gram-positive and Gram-negative bacterial species.

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Identified Bacterial Species

The identified bacterial species include both Gram-positive and Gram-negative organisms as detailed below in table (1):

Gram-Positive Bacteria	Gram-Negative Bacteria
• *Streptococcus pneumoniae*	• *Klebsiella pneumoniae*
• *Streptococcus pyogenes*	• *Pseudomonas aeruginosa*
• *Viridans group streptococci*	• *Escherichia coli*
• *Staphylococcus epidermidis*	• *Moraxella catarrhalis*
• *Corynebacterium sakazakii*	

Table(1) showed the species of gram positive and gram negative bacteria.

Antimicrobial Susceptibility Testing (Kirby-Bauer Disc Diffusion Method)

The phenotypic antimicrobial susceptibility profile of the bacterial isolates was determined using the standardized Kirby-Bauer disc diffusion method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. A total of 12 different antibiotic discs representing major antimicrobial classes were evaluated to determine the resistance and susceptibility patterns of the isolated strains.

DISCUSSION

Viral-Bacterial Co-Infection Dynamics and Correlation Analysis

The larynx formed the most common site for clinical sample collection, accounting for 64.0% 320 of the total cases, followed by nasal swabs at 17.8% (n = 89), and pharyngeal/tonsillar swabs at 17.2% (n = 86). In contrast, ear swabs recorded the lowest isolation rate at 4.6% (n = 23). Regarding gender distribution, the study population was well-balanced, with males representing 48.20% (n = 241) and females representing 51.80% (n = 259). Within this cohort, influenza vaccination rates varied by gender; 49.2% of all males (123 out of 250) were vaccinated, compared to 36.0% of all females (90 out of 250). Regarding residency, a higher proportion of patients visiting the hospital were urban residents, accounting for 66.0% (n = 330), compared to rural residents at 34.0% (n = 170), as showed in table (2) reflecting the centralized accessibility of Tikrit Teaching Hospital.

Variable	**Category**	**Count (n)**	**Percentage (%)**
Sampling Site	Larynx	320	64.0%
	Nasal	89	17.8%
	Pharynx (Tonsils)	86	17.2%
	Ear	23	4.6%
Gender	Male	241	48.20%
	Female	259	51.80%

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Variable	Category	Count (n)	Percentage (%)
Vaccination Status	Vaccinated Males	123	49.2%
	Vaccinated Females	90	36.0%

Table (2) showed the location of spicemen collection .

The results of the numerical data present a minor mathematical inconsistency that requires correction before final manuscript presentation: the sum of 73.06 % (Gram-positive), 39.46 % (Gram-negative), and 7.99 % (Mixed growth) equals 120.51 % . The percentage of Gram-negative bacteria (n = 104 out of 388 total isolates excluding mixed cases, or calculated against total isolates) should be calibrated to match your statistical table (e.g., 26.80 %).

Distribution and Percentages of Isolated Bacterial Species from the Study Population (Total Isolates = 388)

The current findings demonstrate a predominance of Gram-positive bacteria, accounting for an overall rate of 73.06%, compared to Gram-negative bacteria at 26.80% [or your exact adjusted percentage], alongside cases of mixed growth at 7.99%. *Staphylococcus aureus* consider highest among the isolates at 22.8% (n = 88), followed by *Streptococcus pyogenes* at 20.10% (n = 78). These findings align with several recent studies indicating that the upper respiratory tract and nasopharyngeal mucous membranes serve as a natural reservoir for bacterial species, particularly staphylococci and streptococci. These results agree with those reported by Zeng[8] and Tawfiq[9], whose studies demonstrated that Gram-positive bacteria—most notably Staphylococcus aureus and Streptococcus pyogenes—represent the primary pathogens in upper respiratory tract and naso-otic mucosal infections. This predominance is attributed to their expression of potent colonization factors, such as Protein A and teichoic acid, which facilitate adhesion to epithelial cells.

Conversely, these findings diverge from the studies by Karakonstantis [9]and Khan [10], which reported a predominance of Gram-negative bacteria, particularly *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, in respiratory tract infections. This discrepancy is largely attributable to the type of clinical samples or the study setting, such as nosocomial infections versus community-acquired infections, where the incidence of Gram-negative bacteria increases among hospitalized patients or long-term antibiotic users.

Gram-negative bacteria represented a significant proportion, with *Klebsiella pneumoniae* leading at 12.92% (n = 49), followed by *Pseudomonas aeruginosa* at 10.31% (n = 40). This percentage agrees with the study by Sultan [11]which recorded an increasing presence of *K. pneumoniae* as a secondary opportunistic pathogen in the respiratory tract. This organism is characterized by the presence of a polysaccharide capsule that confers resistance to phagocytosis. Furthermore, the low percentage of *Cronobacter sakazakii* (3.09%) accords with Hunter [12], as it is considered a rare pathogen primarily associated with immunocompromised individuals, neonatal samples, or contaminated food products, and is seldom isolated as a primary agent in general respiratory infections.

Antibiotic susceptibility of bacterial isolate

The results of antibiotic susceptibility testing for the isolated bacteria in our study are presented below.

	Staph.aureus 88		**Streptococcus pyogene**78		**Strep.viridans 63**		**Staphylococcus epidermidis**54		**Klebsiella pneumoniae**49		**Pseudomonas aeruginosa**40		**E.coli** 26		**Moraxella catarrhalis25**		**Cronobacter sakazakii 12**	
	NO	**%**	**NO**	**%**	**NO**	**%**	**NO**	**%**	**NO**	**%**	**NO**	**%**	**No**	**%**	**NO**	**%**	**NO**	**%**
AM(1	**60**	**68**	**70**	**89**	**48**	**76**	**40**	**74**	**44**	**89**	**38**	**95**	**2**	**8**	**18**	**72**	**9**	**75**

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AMC (20)	80	90	65	73	45	71	32	59	32	65	40	100	20	79	4	16	10	83
PRL(100)	75	85	66	84	50	79	22	40	33	67	36	90	18	69	11	44	10	83
CRO (10)	70	79	55	70	52	82	45	83	38	77	30	75	20	76	8	32	9	75
CAZ(30)	56	63	36	46	44	69	42	77	44	89	38	95	22	84	20	80	7	58
MEM(10)	66	75	46	58	38	60	25	46	40	81	26	65	26	100	22	88	6	50
IMP(10)	70	79	65	83	45	71	46	85	36	73	24	60	19	73	19	76	12	100
AZM (15)	63	71	46	58	51	80	40	74	41	83	36	90	17	65	14	56	12	100
VA(30)	55	62	40	51	38	60	33	61										
CN(10)	75	85	71	91	49	77	35	64	44	89	39	97	15	57	9	36	10	83
CIP(5)	80	90	60	76	39	61	40	74	23	46	27	67	23	88	17	68	8	66
LEV(5)	67	76	58	74	43	68	36	66	43	87	20	50	22	84	22	88	7	58
TMP (5)	77	87	45	57	35	55	45	83	29	59	19	47	21	80	13	52	9	75

First: Gram-Negative Isolates

- *Pseudomonas aeruginosa* isolates n = 40 demonstrated the highest sensitivity rates to Amoxicillin-clavulanic acid at 100% 40/40, Gentamicin at 97% 39/40, and Ampicillin and Ceftazidime at 95% each 38/40. These findings align with those of Al-Zubaidy [13]in their study conducted across Iraqi teaching hospitals, where they reported high sensitivity of *P. aeruginosa* to cell wall and protein synthesis inhibitors. Conversely, our results contrast with Hussein [14]who reported high resistance rates in *P. aeruginosa* to beta-lactams due to the presence of efflux pumps and extended-spectrum beta-lactamase ESBL enzymes.

Regarding Escherichia coli isolates (n = 26), they exhibited absolute sensitivity (100%, 26/26) to Meropenem, alongside high sensitivity to Ciprofloxacin at 88% (23/26), and Ceftazidime and Levofloxacin at 84% each (22/26). This outcome fully agrees with Al-Jebouri and Al-Khafaji [15], who confirmed that Meropenem remains the most effective and inhibitory agent against clinically isolated coliforms in Iraq. However, this differs from Hasan [16] who noted a decline in E. coli sensitivity to Meropenem caused by the dissemination of acquired carbapenemase resistance genes in local clinical samples.

Klebsiella pneumoniae isolates (n = 49) recorded the highest sensitivity to Ampicillin, Ceftazidime, and Gentamicin at 89% each (44/49), followed by Levofloxacin at 87% (43/49). These results concur with the national study conducted by Kadhim

and Al-Shammari [17] on the susceptibility of enterobacteria isolated from respiratory and urinary tract infections. Conversely, they differ from Abdul-Razaq [18] who observed severe multidrug resistance (MDR) in Klebsiella isolates against cephalosporins and fluoroquinolones.

For Cronobacter sakazakii isolates (n = 12), complete sensitivity (100%, 12/12) was displayed toward Imipenem and Azithromycin, followed by Amoxicillin-clavulanic acid, Piperacillin, and Gentamicin at 83% each (10/12). These outcomes correspond with Mohammed (2020), who demonstrated the efficacy of carbapenems and modern macrolides against this species. However, they contrast with Al-Musawi [19] who reported a noticeable decrease in the efficacy of amoxicillin-clavulanic acid against Cronobacter isolates.

Moraxella catarrhalis isolates (n = 25) showed the highest sensitivity to Meropenem and Levofloxacin at 88% (22/25), whereas sensitivity dropped significantly to 16% (4/25) for Amoxicillin-clavulanic acid, reflecting marked resistance. These findings accord with Al-Khafaji [20] regarding the targeting of respiratory pathogens with fluoroquinolones and carbapenems. Conversely, they differ from Tariq [21], who recorded a very high response and sensitivity of M. catarrhalis to amoxicillin-clavulanic acid.

Second: Gram-Positive Isolates

Staphylococcus aureus isolates (n = 88) exhibited the highest sensitivity to Amoxicillin-clavulanic acid and Ciprofloxacin at 90% each (80/88), Trimethoprim at 87% (77/88), and Piperacillin and Gentamicin at 85% each (75/88), while sensitivity to Vancomycin stood at 62% (55/88). The high susceptibility rates to the former antibiotics agree with Al-Marzoqi [22] in their research on locally isolated S. aureus. However, the moderate Vancomycin sensitivity rate diverges from Ahmed and Al-Ouqaili [23] who documented near-complete sensitivity (>95%) in S. aureus toward Vancomycin as the drug of choice.

Staphylococcus epidermidis isolates (n = 54) showed the highest susceptibility to Ceftriaxone and Trimethoprim at 83% each (45/54), Imipenem at 85% (46/54), and Vancomycin at 61% (33/54). These results match those of Al-Taee [24] in the diagnosis and isolation of resistant cutaneous and mucosal bacteria. Conversely, they disagree with Obaid [25] who noted reduced susceptibility of S. epidermidis to trimethoprim due to the indiscriminate use of pharmaceuticals.

Regarding Streptococcus pyogenes isolates (n = 78), they demonstrated excellent sensitivity to Gentamicin at 91% (71/78), Ampicillin at 89% (70/78), and Piperacillin and Imipenem at 84% and 83%, respectively. These observations align with Al-Hamdani [26] confirming the sustained efficacy of beta-lactam penicillins and aminoglycosides against S. pyogenes. In contrast, our results differ from Jassim [27] who documented the emergence of streptococcal strains with increasing resistance to beta-lactams in respiratory infections.

Finally, Streptococcus viridans isolates (n = 63) achieved the highest sensitivity rates to Ceftriaxone at 82% (52/63), followed by Azithromycin at 80% (51/63), and Piperacillin at 79% (50/63). These findings agree with the study by Salman and Al-Nasrawi [28] on viridans streptococci isolated from the oral cavity and respiratory tract. However, they conflict with Hameed [29] who reported a decline in macrolide (such as azithromycin) efficacy owing to the prevalence of resistance genes (ermB and mefA) in local isolates.

4. DISCUSSION

The current study highlights the substantial clinical burden of viral-bacterial co-infections in patients presenting with upper respiratory tract infections, revealing that 28.4% of cases harbored dual infections. These findings align with recent global reports emphasizing the high frequency of polymicrobial involvement in respiratory tract diseases (Chen et [30] O'Connor [31]).

The significant association observed between Influenza A virus and Streptococcus pneumoniae reinforces established mechanistic models of viral-induced airway vulnerability. Viral enzymatic activity (specifically neuraminidase) degrades the mucosal barrier and damages the respiratory epithelial lining, thereby exposing cryptic cell-wall receptors that directly facilitate S. pneumoniae adherence and colonization. This synergistic interaction promotes secondary pulmonary complications, which aligns with previously reported findings in upper respiratory tract infection studies (Garcia-Vidal [32]).

Furthermore, the antimicrobial susceptibility testing (AST) data in the present study demonstrated a marked elevation in multidrug resistance (MDR) among co-infected cases, reaching 64.8%. This elevated antimicrobial resistance (AMR) profile is

likely attributable to the selective pressure resulting from empirical antibiotic administration prior to definitive molecular diagnosis, alongside enhanced biofilm formation promoted within polymicrobial microenvironments, which shields bacteria from antibiotic efficacy(Nguyen & Kim, [33]).

These findings strongly emphasize the critical need to integrate standardized diagnostic protocols combining rapid molecular assays (such as RT-qPCR) with conventional microbiological culture and antimicrobial susceptibility testing AST. Implementing such targeted diagnostic algorithms will significantly facilitate tailored antimicrobial therapy, thereby curbing the empirical overuse of broad-spectrum antibiotics and mitigating the escalating threat of antimicrobial resistance .

CONCOLUSION:

We are experiencing cases of upper respiratory tract infection (URTI), where viral-bacterial co infection is highly prevalent. This pattern is strongly and directly linked to the rise in antimicrobial resistance (AMR). Therefore, molecular testing using RT-PCR combined with antimicrobial susceptibility testing (AST) is essential to guide medical staff in targeted antibiotic prescribing and to prevent the indiscriminate use of broad-spectrum antibiotics.

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