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Estimation the Pathogenesis of Enterobacter cloacae isolated from High Vaginal Swab in Pregnant Women

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Abstract

Any opportunistic Gram-negative pathogen, Enterobacter cloacae with key epidemiological features such as acquisition of antimicrobial resistance along with high adhesion to a surface leading to biofilms. To isolate and characterization of E.cloacae from High vaginal swab (HVS); to determine its antimicrobial susceptibility pattern, biofilm forming ability and catheter adherence. Samples of HVS were processed and E. cloacae was isolated in 4% of samples confirmed by VITEK® 2 system. Antimicrobial susceptibility testing revealed that all isolates were highly susceptible to most of antibiotics, particularly meropenem, gentamicin, tobramycin and many cephalosporins and least susceptible to ciprofloxacin Biofilm thickness quantification showed that isolates were able to produce biofilm in mixed populations ranging from weak to strong producers. In addition, catheter adhesion testing demonstrated strong adherence ability in all isolates, with high adhesion index values (9.47–12.60). In conclusion, although E. cloacae was infrequently isolated from HVS samples, the isolates exhibited significant virulence traits, including biofilm formation and strong adhesion, which may contribute to persistence and clinical relevance in gynecological infections.

Keywords: *HVS, Enterobacter cloacae, susceptibility test, biofilm formation, catheter adhesion.*

1. Introduction

As a typical element of the natural flora of the human gastrointestinal system, Enterobacter cloacae is an opportunistic Gram-negative member of the Enterobacterales family. But it has become a significant nosocomial & opportunistic pathogen that may cause a variety of illnesses, especially in individuals with impaired immune systems. Its exceptional capacity to acquire several antimicrobial resistance mechanisms, particularly AmpC β-lactamases and extended-spectrum β-lactamases (ESBLs), which lead to treatment failure and restricted therapeutic alternatives, is primarily responsible for its clinical significance (Mezzatesta et al., 2012; Davin-Regli&Pagès, 2015).

Recent evidence indicates that E. cloacae is no longer restricted to intestinal or hospital-associated infections but is increasingly isolated from diverse clinical specimens, including urinary tract, respiratory tract, bloodstream, and genital tract samples. The isolation of E. cloacae from HVS is clinically relevant because it might indicate a disturbance of the normal vaginal microbiota, characterized by Lactobacillus dominance that could lead to increased inoculation or infection (Sobel, 2000; Linhares et al., 2021). This imbalance is commonly associated with antibiotic consumption, hormonal alteration, hospitalization or any other cases of immunosuppression.

E. cloacae's capacity to cling to human epithelium and abiotic tissues in order to form biofilms is one of the many virulence variables that are closely linked to its pathogenicity. The formation of biofilm greatly contributes to bacterial persistence, antibiotic resistance, and evasion of the host immune response. Emerging studies have identified a correlation between E. cloacae biofilm formation on biological surfaces and medical devices by persistent and reoccurring infections (Misra et al., 2022; Liu et al., 2022; Cangui-Panchi et al., 2022). Early steps of colonization and infection are also represented by catheter and surface adhesion. Fimbriae, outer membrane proteins and hydrophobic interactions (the mechanisms that allow bacteria to stick to inert surfaces like catheters). The systematic review of catheter-associated infections indicated that 59–100% of clinical isolates are able to biofilm formation, establishing the clinical importance of adhesion/persistence mechanisms in device-associated infections (Cangui-Panchi et al., 2022; Stepanović et al., 2020) Most previous studies investigated infections of the bloodstream or urinary tract and there is a very large gap in data concerning its role in vaginal colonization/infection (Flores-Mireles et al., 2020; Liu et al., 2022). Thus, the present study hypothesize that E. cloacae isolates from HVS harbor virulence potential evidenced by high biofilm formation and adherence capabilities as well as heterogeneous antimicrobial resistance patterns.

This study aims at isolation and identification of E. cloacae from high vaginal swabs along with antimicrobial susceptibility, biofilm formation and catheter adhesion to understand its virulence potential and clinical significance in gynecological infections.

Material and Method

Isolation of Enterobacter cloacae

One hundred high vaginal swabs (HVS) from pregnant patients who visited the outpatient clinic were collected for this investigation. All patients were clinically examined by a specialist physician, and samples were obtained under aseptic conditions for microbiological investigation. The specimens were cultivated on several selective and differential media, such as MacConkey agar and Blood agar, and then incubated under suitable conditions. Colony morphology, Gram staining, and biochemical traits were used for preliminary identification. The VITEK® 2 system (bioMérieux, France), which offered automated and precise biochemical identification, was used to further validate the diagnosis of *E. cloacae*.

Estimation of the pathogenesis

1-The antibiotic susceptibility

The Kirby Bauer technique was used to determine the microorganisms' susceptibility to antibiotics. This method was used to determine the susceptibility of bacteria to Netilmicin (Net), Ofloxacin (OF), Meropenem (MEM), Chloramphenicol (C), Ciprofloxacin (CIP), Cefepime (CPM), Levofloxacin (LE), , Tobramycin (TOB), Norfloxacin (NOR) ,Amikacin (AK), Cefoxitin (FOX), Nalidixic acid (NA), and Gentamicin (CN) Cefuroxime (CXM), Tetracycline (TE) and the result was interpreted according to the guideline of CLSI.

2-Biofilm formation

The microtiter plate technique was used to evaluate the bacterial isolates' capacity to generate biofilm. In short, new bacterial colonies were added to Tryptic Soy Broth (TSB) and allowed to incubate for 18 to 24 hours at 37°C. Following a 1:100 dilution of the overnight cultures in fresh TSB, 200 µL of the suspension was added to sterile 96-well flat-bottom microtiter plates. To enable the production of biofilms, the plates were incubated at 37°C for a whole day in static circumstances. Following incubation, planktonic cells were extracted, and non-adherent cells were gently washed out of the wells using phosphate-buffered saline

(PBS). A 0.1% crystal violet solution was used to fix and stain the adhering biofilm for ten to fifteen minutes. The surplus is washed away using distilled water, and the color linked to ethanol is dissolved. According to Stepanović et al. (2000), isolates were classified as non, weak, moderate, or strong biofilm producers based on the optical density (OD) of each well measured at a wavelength of 570 nm using a microplate reader.

$OD \leq ODc \rightarrow$ Non producer

$ODc < OD \leq 2 \times ODc \rightarrow$ Weak

$2 \times ODc < OD \leq 4 \times ODc \rightarrow$ Moderate

$OD > 4 \times ODc \rightarrow$ strong

3- Catheter Adhesion Assay

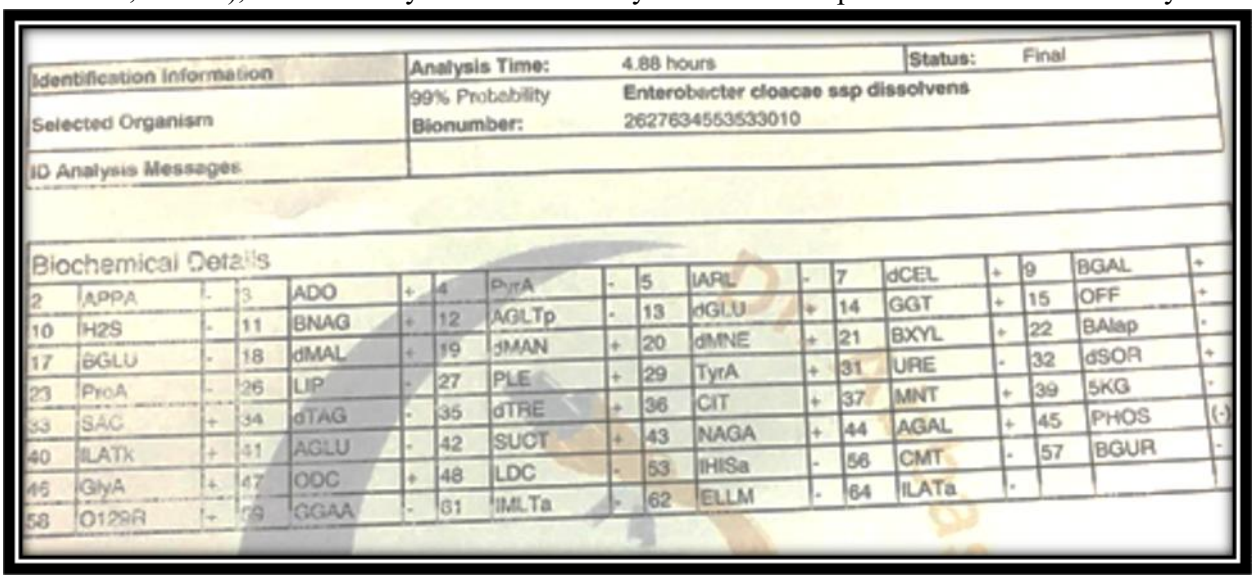
The ability of bacterial isolates to stick to catheters was assessed using the catheter adhesion test. To cultivate suspended bacterial isolates in Tryptic Soy Broth (TSB), sterilized Foley catheter segments were incubated for 24–48 hours at 37°C (1–2 cm). After incubation, non-adherent cells were removed from catheter segments by rinsing them with phosphate-buffered saline (PBS). The remaining adhered bacteria were then stained using 0.1% crystal violet, as previously mentioned. After the bound dye was dissolved, optical density (OD570 nm) was measured to determine the degree of bacterial adherence. The results showed that most of the tested isolates displayed variation in adherence capacity ability suggesting a variation with regards to the growth on catheter surface and biofilm formation. Adhesion Index (AI) (Christensen et al., 1985): Higher OD values are considered as vigorous whereas lower ones reflect weak or limited adhesion.

$$\text{Adhesion Index (AI)} = \frac{OD_{sample}}{OD_{control}}$$

Results and Discussion

Isolation and identification

Bacterial species isolated were 4 (4%) Enterobacter cloacae whereas in the rest of 96% samples; different types of bacteria were isolated. Enterobacter cloacae was further identified using the VITEK® 2 technology (bioMérieux, France), which swiftly and automatically identified the specific biochemical identity.



Identification Information		Analysis Time:	4.88 hours	Status:	Final
Selected Organism		99% Probability	Enterobacter cloacae ssp dissolvens		
		Bionumber:	2627634553533010		
ID Analysis Messages					

Biochemical Details

2	APPA	-	3	ADO	+	4	PyrA	-	5	IARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	+	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	+	44	AGAL	+	45	PHOS	(-)
46	GlyA	+	47	ODC	+	48	LDC	-	53	IHISa	-	56	CMT	-	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

Figure (1): VITEK® 2 system ,*E.cloacae*

In contrast, only 4% of high vaginal swab (HVS) specimens isolated *E.cloacae* in the current study with other bacterial species grown for 96% in total. The low prevalence, in particular, was in line with E. cloacae being a non-dominant member of the normal vaginal microbiota and more an opportunistic pathogen rather than a true vaginal typing colonizer. Therefore, the identification of C. albicans to formulate vaginal specimens might reflect transient colonization or infection and probably variations in the conventional Lactobacillus-dominated vaginal flora (Sobel, 2000; Linhares et al., 2021). The low isolation rate may possibly be a result of E. cloacae's biased ecological niche, which is linked with nosocomial or device-related infections and inhabits the gastrointestinal system rather than being mostly genitourinary. Its presence detected in HVS samples might be associated with risk factors, such as previous administration of antibiotics, being hospitalized or due perhaps to an immunological break down and pre-disposal to opportunistic pathogens colonizing atypical sites (Davin-Regli&Pagès 2015).

The diagnosis of *E. cloacae* was confirmed using the VITEK® 2 automated system, a widely used biochemical technique renowned for its quick, precise, and consistent detection of Gram-negative bacteria. Compared to traditional biochemical techniques, the use of automated systems like VITEK® 2 increases diagnostic reliability, especially when distinguishing members of the *E.cloacae* complex, which frequently share phenotypic traits (Mezzatesta et al., 2012; O'Hara et al., 2020).

Susceptibility test

The Sensitivity rate of Netilmicin (Net) 75%, Ofloxacin (OF) 50%, Meropenem (MEM) 100%, Chloramphenicol (C) 50%, Ciprofloxacin (CIP) 25%, Levofloxacin (LE) 75%, Amikacin (AK) 50%, Tobramycin (TOB) 100%, Norfloxacin (NOR) 100%, Tetracycline (TE) 100%, Cefuroxime (CXM) 100%, Cefepime (CPM) 100%, Cefoxitin (FOX) 100%, and Nalidixic acid (NA) 75%, and Gentamicin (CN) 100%.

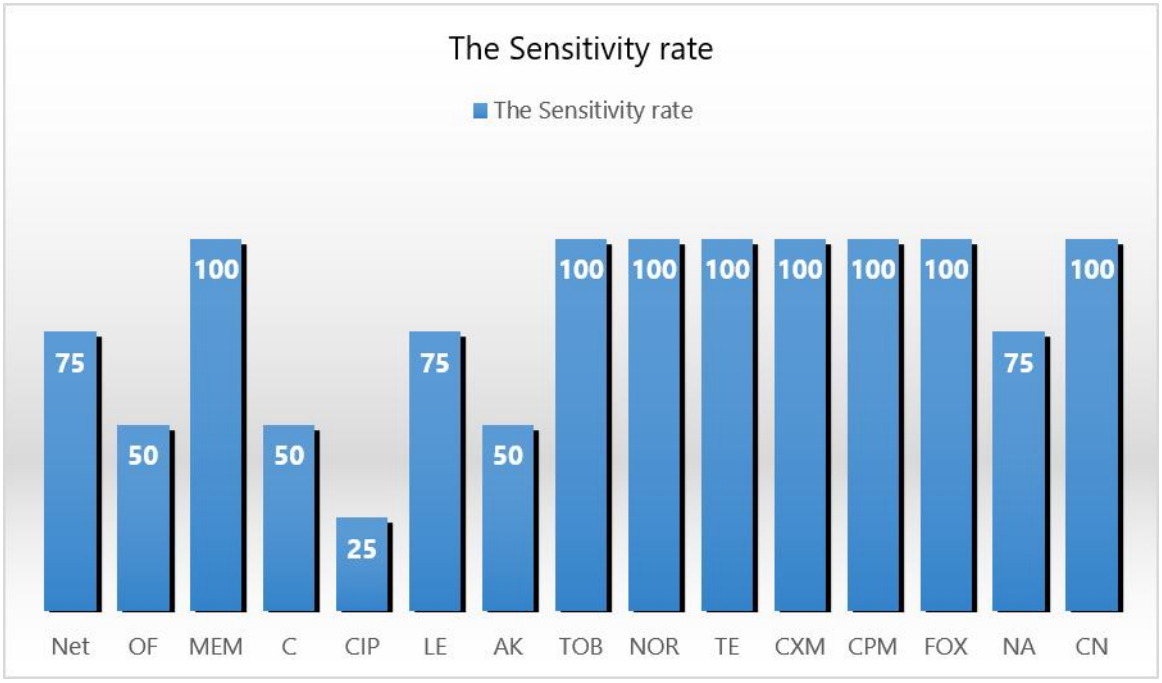


Figure (2): The Sensitivity rate of *E. cloacae*

The antimicrobial susceptibility pattern of Enterobacter cloacae isolated from high vaginal swabs (HVS) in the present study shows both agreement and variation when compared with previous studies involving vaginal and .swab-derived isolatesEnterobacter species are among the organisms isolated from vaginal samples and showed high susceptibility to broad-spectrum antibiotics like carbapenems, according to a study looking into aerobic

bacteria from high vaginal swabs. This finding supports the current finding of complete sensitivity to meropenem (100%) (Ahmed et al., 2006).

Similarly, surveillance studies including isolates from vaginal and other clinical swab specimens showed that *E. cloacae* maintains high susceptibility to aminoglycosides, which is consistent with the present results for gentamicin and tobramycin 100% (Tschudin-Sutter et al., 2023).

Nonetheless, this study's decreased susceptibility to fluoroquinolones like ciprofloxacin (25%) is consistent with resistance patterns found in urogenital and clinical isolates, where rising resistance is linked to both plasmid-mediated quinolone resistance and mutations in the gyrA and parC genes (Jacoby, 2005). In addition, as reported by several studies, the E. cloacae isolated from clinical samples including swabs tend to develop multidrug resistance mediated by extended-spectrum β-lactamases (ESBLs) and AmpC β-lactamases that significantly diminish β-lactams antibiotics efficacy (Harris et al., 2015; Davin-Regli&Pagès, 2015). In contrast, the hyper susceptibility to cephalosporins observed in this study was unexpected, since E. cloacae is known to possess inducible AmpC enzymes that confer resistance. This difference may be attributed to local epidemiology, differences in antibiotic pressure, or the absence of derepressed mutants in isolates from the vaginal niche.

Biofilm formation

The examined samples varied in the Enterobacter cloacae isolates' capacity to create biofilms. Isolate 1 was weak biofilm producer through its ability to adherence. Biofilm formation moderate for isolate 2 showed it can develop organized biofilm. Stated otherwise, Isolates 2,3 and 4 produced significant biofilms as in table (1).

Table (1): Biofilm Formation of Enterobacter cloacae Isolates

Isolate No.	OD570 Value	Biofilm Formation Ability
***E. cloacae* 1**	**0.21**	**Weak biofilm producer**
***E. cloacae* 2**	**1.34**	**Strong biofilm producer**
***E. cloacae* 3**	**1.78**	**Strong biofilm producer**
***E. cloacae* 4**	**2.59**	**Strong biofilm producer**

It was discovered that Enterobacter cloacae isolates from HVS in the current investigation differed in their capacity to create biofilms between weak and strong producers. This finding is consistent with other previous studies of clinical and swab-derived isolates. A study by Misra et al. (2022) showed that *E. cloacae* biofilms are structurally complex and genetically heterogeneous between strains which is consistent with the differences in EPS production and gene regulation so far observed from the present isolates. Likewise, Liu et al. (2022) performed a clinical cluster analysis demonstrated variation in the biofilm-forming capacity between E. cloacae isolates from human infections, which correlates with the presence of virulence genes and adaptation to environmental niches, supported by the strong biofilm producers achieved in isolates 2, 3, and 4. In addition, Qian et al. (2020) Carbapenem-resistant *E. cloacae* was also shown to have increased biofilm production, which has been linked to persistence and antibiotic resistance, reinforcing the clinical relevance of the very-high biofilm strength organisms we identified in this study. Furthermore, Liu et al. (2020) showed that *E. cloacae* biofilm formation can substantially vary based on metabolic regulation and extracellular polymeric substance production, which accounts for both the low and moderate biofilm producers detected among HVS isolates.

Catheter Adhesion Assay

All Enterobacter cloacae isolates had a very high adherence capacity to catheter surfaces, as determined by catheter adhesion assay. The adhesion indices (AI) were dramatically high because the values of addition-optical density (OD570) for all tested isolates were higher than negative control (OD = 0.150). The AI ranged from 9.47 to 12.60, indicating that all isolates exhibited strong adhesion ability. Specifically, the highest adhesion index is E. cloacae 3 (12.60), and the lowest is E. cloacae 1 (9.47) and as in table (2).

Table (2): Catheter Adhesion Assay

Isolate	OD570 Value	OD Control	Adhesion Index (AI)	Interpretation
***E. cloacae* 1**	1.420	0.150	9.47	Strong adhesion
***E. cloacae* 2**	1.610	0.150	10.73	Strong adhesion
***E. cloacae* 3**	1.890	0.150	12.60	Strong adhesion
***E. cloacae* 4**	1.750	0.150	11.67	Strong adhesion

The results of the catheter adhesion assay for all *E.cloacae* isolates from HVS showed a significant ability of all isolate to adhere on catheter surfaces with an AI value range 9.47-12.60. This suggests a high ability to colonize surfaces, an important virulence factor in medical device-associated infections.

A study by Bhardwaj et al. (2021) *E. cloacae* has been shown to strongly adhere to the urinary catheter materials due as it possesses surface-associated proteins and fimbrial structures that enable initial attachment. Similarly, Hennequin et al. (2020) isolated Enterobacter species from clinical specimens and showed strong adhesion activity to abiotic surfaces, likely explaining the high indices of adhesion found in this study.

In addition, Flores-Mireles et al. (2020) emphasized that the adherence of bacteria to surfaces of catheters is an important initial step in device-associated infections, mediated by adhesins and hydrophobic interactions, which together account for the high levels of adhesion seen among all isolates. Furthermore, Jacobsen et al. (2021) demonstrated that urogenital Enterobacter ales species rapidly colonize catheter surfaces with variable adhesion strength in an expression-based manner among strains.

Variations in adhesion index (higher for isolate 3 and lower for isolate 1) may result from differences in surface protein expression and fimbrial density (bacteria-adherence related factors) as well as interactions with catheter materials. Overall, our results show that E. cloacae isolates from HVS have a great ability to adhere to catheters and this adhesion makes these pathogens more susceptible to continuous device-associated infections. However, Liu et al. (2020) Lactic acid can prevent biofilm and bacterial adhesion, suggesting that natural vaginal microbiome components could inhibit E. cloacae colonization potential.

Conclusion

Although isolation rates of E. cloacae from HVS samples is low, the isolates contain important virulence factors such as biofilm formation and strong adhesion ability (AA) which may play a role in persistence and clinical significance of these organisms in gynecological infections. This factor contributes to bacteria in colonization of both mucosal and abiotic surfaces, increases the resistance against host innate defenses and antimicrobial agents, and may facilitate recurrent or chronic infection affecting patients using indwelling medical devices (e.g., urinary catheters).

References

1. Ahmed, S., et al. (2006). Frequency and antimicrobial susceptibility of aerobic bacterial vaginal isolates. Journal of the College of Physicians and Surgeons Pakistan, 16(3), 196–199.
2. Bhardwaj, N., et al. (2021). Bacterial adhesion mechanisms on urinary catheter surfaces and role of surface proteins in Enterobacter spp. Journal of Medical Microbiology, 70(4), 001345.
3. Cangui-Panchi, S. P., et al. (2022). Biofilm-forming microorganisms causing hospital-acquired infections from intravenous catheter: A systematic review. Current Research in Microbial Sciences, 3, 100175.
4. Christensen, G. D., Simpson, W. A., Younger, J. J., Baddour, L. M., Barrett, F. F., Melton, D. M., & Beachey, E. H. (1985). *Adherence of coagulase-negative staphylococci to plastic tissue culture plates:*

a quantitative model for the adherence of staphylococci to medical devices. Journal of Clinical Microbiology, 22(6), 996–1006.

5. Davin-Regli, A.,&Pagès, J. M. (2015). Enterobacter aerogenes and Enterobacter cloacae; versatile bacterial pathogens confronting antibiotic treatment. Frontiers in Microbiology, 6, 392.
6. Davin-Regli, A.,&Pagès, J. M. (2015). Enterobacter cloacae complex: Clinical impact and emerging antibiotic resistance. Frontiers in Microbiology, 6, 392.
7. Davin-Regli, A.,&Pagès, J. M. (2015). Enterobacter cloacae complex: Clinical impact and emerging antibiotic resistance. Frontiers in Microbiology, 6, 392.
8. Flores-Mireles, A. L., et al. (2020). Urinary catheter-associated biofilm formation and infection. Nature Reviews Microbiology, 17(6), 348–364.
9. Flores-Mireles, A. L., et al. (2020). Urinary catheter-associated biofilm formation and bacterial adhesion mechanisms. Nature Reviews Urology, 17(6), 348–364.
10. Harris, P. N. A., et al. (2015). Treatment of infections caused by Enterobacteriaceae with inducible AmpC β-lactamases. Clinical Microbiology Reviews, 28(3), 646–689.
11. Hennequin, C., et al. (2020). Adhesion properties of Enterobacter cloacae complex clinical isolates on abiotic surfaces. Infection, Genetics and Evolution, 80, 104185.
12. Jacobsen, S. M., et al. (2021). Surface-associated virulence factors in Enterobacterales from clinical infections. Frontiers in Microbiology, 12, 672890.formation of Enterobacter cloacae. Food Science, 41(7), 1–7.
13. Jacoby, G. A. (2005). Mechanisms of resistance to quinolones. Clinical Infectious Diseases, 41(Suppl 2), S120–S126.
14. Linhares, I. M., et al. (2021). Vaginal microbiota and infections. Revista de Ginecologia e Obstetrícia, 43(2), 85–92.
15. Linhares, I. M., et al. (2021). Vaginal microbiota and infections. Revista Brasileira de Ginecologia e Obstetrícia, 43(2), 85–92.
16. Liu, S. L., Chen, L. Q., et al. (2022). Cluster differences in antibiotic resistance, biofilm formation, mobility, and virulence of clinical Enterobacter cloacae complex. Frontiers in Microbiology, 13, 814831.
17. Liu, S. L., et al. (2022). Cluster differences in antibiotic resistance, biofilm formation, and virulence of Enterobacter cloacae complex. Frontiers in Microbiology, 13, 814831.
18. Mezzatesta, M. L., et al. (2012). Enterobacter cloacae complex: clinical impact and resistance. Future Microbiology, 7(7), 887–902.
19. Mezzatesta, M. L., Gona, F.,&Stefani, S. (2012). Enterobacter cloacae complex: Clinical impact and emerging antibiotic resistance. Future Microbiology, 7(7), 887–902.
20. Misra, T., et al. (2022). Insights into biofilm dynamics of Enterobacter cloacae. Frontiers in Microbiology, 13, 877060.
21. Misra, T., Tare, M. T.,&Jha, P. N. (2022). Insights into the dynamics and composition of biofilm formed by Enterobacter cloacae. Frontiers in Microbiology, 13, 877060.
22. O'Hara, C. M., et al. (2020). Update on the identification of Enterobacter cloacae complex. Journal of Clinical Microbiology, 58(5), e01435-19.
23. Qian, W., Liu, M., Fu, Y., Wang, T., Zhang, J., Yang, M., Sun, Z., Li, X.,&Li, Y. (2020). Antimicrobial and antibiofilm activities of citral against carbapenem-resistant Enterobacter cloacae. Foodborne Pathogens and Disease, 17(7), 459–465.
24. References (APA style)
25. Sobel, J. D. (2000). Bacterial vaginosis and vaginal infections. Clinical Infectious Diseases, 31(2), 398–

402.
26. Sobel, J. D. (2000). Bacterial vaginosis and vaginal infections. Clinical Infectious Diseases, 31(2), 398–402.
27. Stepanović, S., et al. (2020). Biofilm formation on medical devices. Journal of Hospital Infection, 104(3), 299–305.
28. Stepanović, S., Vuković, D., Hola, V., Di Bonaventura, G., Djukić, S., Cirković, I., & Ruzicka, F. (2000). *A modified microtiter-plate test for quantification of staphylococcal biofilm formation*. Journal of Microbiological Methods, 40(2), 175–179. https://doi.org/10.1016/S0167-7012(00)00122-6
29. Tschudin-Sutter, S., et al. (2023). Antimicrobial resistance patterns of Enterobacter cloacae from clinical specimens. Antibiotics, 12(4), 673.