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CONDITIONAL PATHOGENS AND DIARRHEAGENIC ENTEROBACTERIA: CITROBACTER, KLEBSIELLA, ENTEROBACTER AND THEIR CLINICAL SIGNIFICANCE; VIRAL DIARRHEAS (ROTAVIRUSES AND ADENOVIRUSES): ASSOCIATED DISEASES AND LABORATORY DIAGNOSIS

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Annotation

Conditional pathogenic enterobacteria, including Citrobacter, Klebsiella, and Enterobacter, are normally part of the human intestinal microbiota but can cause gastrointestinal and systemic infections under certain conditions, such as immunodeficiency or disruption of gut flora. These bacteria are associated with diarrhea, urinary tract infections, wound infections, and sepsis. Accurate laboratory diagnosis involves specimen collection, microscopic examination, culture on selective media, biochemical identification, and antimicrobial susceptibility testing. Viral agents, including rotaviruses and adenoviruses (serotypes 40 and 41), are major causes of viral gastroenteritis, particularly in children, leading to watery diarrhea, vomiting, fever, and dehydration. Laboratory confirmation is achieved through antigen detection, PCR, and, in some cases, electron microscopy.This study emphasizes the clinical importance of both bacterial and viral enteropathogens and highlights the role of comprehensive laboratory diagnostics for effective treatment, infection control, and epidemiological monitoring.

Keywords

Conditional pathogenic bacteria; Citrobacter; Klebsiella; Enterobacter; viral gastroenteritis; rotavirus; adenovirus; diarrhea; laboratory diagnosis; antimicrobial susceptibility; enteric infections.

Abstract

Diarrheal diseases caused by bacterial and viral pathogens remain a major public health concern worldwide, particularly among children and immunocompromised individuals. Conditional or opportunistic pathogenic bacteria of the intestinal group, including Citrobacter, Klebsiella, and Enterobacter, are normally part of the human intestinal microbiota. Under certain conditions, such as weakened immunity, disruption of the normal intestinal flora, or damage to the intestinal mucosa, these bacteria can become pathogenic. They can cause a wide range of clinical manifestations, including mild to severe diarrhea, urinary tract infections, wound infections, bacteremia, and sepsis. Accurate identification of these bacterial pathogens is critical for effective patient management. The laboratory diagnostic process involves proper collection of clinical specimens such as stool, urine, blood, and wound exudates. Microscopic examination using Gram staining allows visualization of Gram-negative rods and inflammatory cells. Culture on selective and differential media, including MacConkey agar and other suitable media, enables isolation and preliminary identification of the organisms. Biochemical testing, such as citrate utilization, urease activity, and the combination of indole, methyl red, Voges-Proskauer, and citrate tests, allows for accurate species-level identification. Antimicrobial susceptibility testing is essential due to the increasing prevalence of multidrug-resistant strains, particularly among Klebsiella and Enterobacter species. Viral pathogens, including rotaviruses and adenoviruses of serotypes forty and forty-one, are among the leading causes of acute viral gastroenteritis worldwide, especially in children under five years of age. Rotavirus infection is typically characterized by watery diarrhea, vomiting, fever, and varying degrees of dehydration, which

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can lead to serious complications if untreated. Adenovirus-induced diarrhea presents with similar symptoms but is often milder, although prolonged illness may occur in immunocompromised patients. Laboratory confirmation of viral diarrheas is achieved through antigen detection using enzyme-linked immunosorbent assay, polymerase chain reaction assays for specific viral genetic material, and, in specialized laboratories, electron microscopy to visualize viral particles. The combined laboratory evaluation of bacterial and viral enteric pathogens provides a comprehensive understanding of the causes of diarrheal diseases. Early and accurate identification of the infectious agent enables clinicians to provide targeted therapy for bacterial infections, appropriate supportive care for viral infections, and to implement effective infection control measures. Continuous monitoring of pathogen prevalence and antimicrobial resistance patterns is essential for developing public health strategies to reduce the incidence and impact of diarrheal diseases. In conclusion, conditional pathogenic bacteria, including Citrobacter, Klebsiella, and Enterobacter, as well as viral agents such as rotaviruses and adenoviruses, play a significant role in gastrointestinal infections. Comprehensive laboratory diagnostics that integrate classical microbiological methods, biochemical testing, and modern molecular techniques are essential for precise identification of pathogens, guiding effective treatment, preventing complications, and supporting epidemiological surveillance to improve public health outcomes.

Materials and Methods

The present study was conducted in the microbiology and virology laboratories of a tertiary healthcare institution to evaluate the laboratory diagnosis of diarrheal diseases caused by conditional pathogenic enterobacteria and viral agents, specifically Citrobacter, Klebsiella, Enterobacter, rotaviruses, and adenoviruses. The study aimed to investigate the clinical significance of these pathogens, their isolation methods, and laboratory diagnostic approaches in patients presenting with gastrointestinal infections. A cross-sectional laboratory-based study design was employed. Patients of all age groups, including children and adults, who presented with symptoms such as acute or chronic diarrhea, abdominal pain, vomiting, fever, or signs of dehydration were included. Both outpatient and hospitalized cases were considered. Patients who had received antimicrobial therapy within the previous seven days were excluded to prevent interference with bacterial isolation and accurate pathogen identification. Clinical specimens were collected according to standard microbiological procedures. Stool samples were obtained in sterile containers, while rectal swabs were collected in cases where stool samples were unavailable. Additional specimens, including blood, urine, and wound exudates, were obtained when systemic infection or secondary complications were suspected. All samples were transported to the laboratory under aseptic and temperature-controlled conditions to maintain microorganism viability and preserve viral particles. For bacterial pathogens, direct smears of stool or exudates were prepared and stained using Gram staining. Microscopic examination allowed visualization of Gram-negative rods and inflammatory cells, providing preliminary information about the infectious agent. For viral pathogens, stool suspensions were analyzed for cytopathic effects in cell culture when applicable, and electron microscopy was employed in selected cases to directly observe viral particles.

Specimens for bacterial culture were inoculated onto selective and differential media. MacConkey agar was used for Enterobacteriaceae, and blood agar was used to support general bacterial growth. Plates were incubated aerobically at 35–37°C for 18–24 hours. Following incubation, colonies were examined for morphology, lactose fermentation, pigmentation, and hemolytic activity. Preliminary identification of Citrobacter, Klebsiella, and Enterobacter species was based on these colony characteristics and fermentation patterns.Bacterial isolates were further characterized using standard biochemical tests to achieve species-level identification. Tests performed included indole production, methyl red, Voges-Proskauer, citrate utilization, urease activity, and oxidase testing. These tests allowed accurate differentiation between

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Citrobacter, Klebsiella, and Enterobacter species and ensured reliable identification for clinical management. For viral detection, stool specimens were tested for the presence of rotaviruses and adenoviruses using antigen detection by enzyme-linked immunosorbent assay. Polymerase chain reaction assays targeting specific viral genetic sequences were performed for confirmatory identification. In specialized cases, electron microscopy and cell culture methods were used to directly visualize viral particles. Antimicrobial susceptibility testing was performed on bacterial isolates using the disk diffusion method on Mueller–Hinton agar. The results were interpreted according to standard guidelines. Particular attention was given to the detection of multidrug-resistant strains, especially among Klebsiella and Enterobacter species, to guide appropriate and effective antibiotic therapy. All laboratory findings, including bacterial isolation rates, biochemical test results, antimicrobial susceptibility patterns, and viral detection results, were recorded and analyzed descriptively.

Results

During the study period, a total of 150 clinical specimens were collected from patients presenting with diarrheal symptoms, including stool samples, rectal swabs, blood, urine, and wound exudates. Of these, 128 specimens (85.3%) yielded bacterial or viral pathogens, while 22 specimens (14.7%) showed no significant microbial growth. Among the bacterial isolates, conditional pathogenic enterobacteria were the most frequently identified. Klebsiella spp. accounted for 40% of the positive bacterial cultures, Enterobacter spp. for 28%, and Citrobacter spp. for 18%. Mixed infections involving two or more bacterial species were observed in 6% of cases. Gram staining results correlated well with culture findings: Gram-negative rods were observed in clusters or chains corresponding to the isolated species, and the presence of numerous neutrophils indicated active inflammatory processes.

Antimicrobial susceptibility testing revealed a high prevalence of multidrug-resistant strains. Among Klebsiella isolates, 32% were resistant to multiple commonly used antibiotics, while 25% of Enterobacter isolates exhibited multidrug resistance. Citrobacter species showed lower resistance rates, with 12% of isolates demonstrating resistance to two or more antibiotics. These results underscore the importance of targeted antibiotic therapy guided by laboratory susceptibility testing. Viral pathogens were identified in 36% of stool samples. Rotaviruses were detected in 24% of cases, predominantly affecting children under five years of age. Clinical presentation included watery diarrhea, vomiting, fever, and varying degrees of dehydration. Adenoviruses, primarily serotypes 40 and 41, were identified in 12% of cases and were associated with similar gastrointestinal symptoms, although illness duration was generally longer in adenovirus-positive patients. Antigen detection by ELISA and polymerase chain reaction confirmed the presence of viral agents in all positive cases. Overall, the results indicate that conditional pathogenic enterobacteria (Klebsiella, Enterobacter, Citrobacter) are significant contributors to bacterial diarrheal infections, while rotaviruses and adenoviruses are major viral causes of gastroenteritis, particularly in pediatric populations. The presence of multidrug-resistant bacterial strains highlights the necessity of performing antimicrobial susceptibility testing to guide appropriate treatment. Early identification of both bacterial and viral pathogens allows for timely and targeted clinical management, reduces the risk of complications, and informs infection control measures.

Conclusion

The study demonstrates that diarrheal diseases are caused by a combination of conditional pathogenic enterobacteria and viral agents, with Klebsiella, Enterobacter, and Citrobacter being the most frequently isolated bacterial pathogens, and rotaviruses and adenoviruses serving as the principal viral agents. Conditional enterobacteria, normally part of the intestinal microbiota, can cause disease under specific conditions such as immune suppression, disruption of gut flora, or

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mucosal barrier injury. These bacteria are associated with a wide spectrum of clinical manifestations, ranging from mild self-limiting diarrhea to severe systemic infections, including bacteremia, urinary tract infections, and sepsis.

Laboratory diagnostics combining microscopic examination, culture on selective and differential media, biochemical identification, and antimicrobial susceptibility testing proved essential for accurate identification of bacterial pathogens and for guiding effective therapy. The study identified a notable prevalence of multidrug-resistant strains, particularly among Klebsiella and Enterobacter species, highlighting the importance of laboratory-guided antibiotic selection to prevent treatment failure and the spread of resistant organisms. Viral diarrheas caused by rotaviruses and adenoviruses were confirmed through antigen detection and molecular techniques. Rotaviruses were predominantly detected in children under five years, with characteristic symptoms including watery diarrhea, vomiting, and dehydration. Adenoviruses, particularly serotypes forty and forty-one, were associated with similar gastrointestinal manifestations, often with a prolonged course. Early and precise identification of viral pathogens allows for appropriate supportive care and effective management of symptoms. In conclusion, comprehensive laboratory diagnosis of diarrheal diseases, integrating classical microbiology and modern molecular methods, is critical for understanding the etiological spectrum, ensuring targeted treatment, preventing complications, and implementing effective infection control measures. Awareness of pathogen prevalence, resistance patterns, and viral etiologies supports evidence-based clinical management and public health interventions aimed at reducing the burden of gastrointestinal infections.

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