

IN VITRO EVALUATION OF THE EFFECTS OF IODINATED CONTRAST AGENTS ON GUT MICROBIOTA

İn Vitro İyotlu Kontrast Maddelerin Bağırsak Mikroorganizmaları Üzerindeki Etkilerinin Değerlendirilmesi

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ABSTRACT

Objective: The aim of this study was to investigate whether commonly used iodinated contrast agents exert bacteriostatic or bactericidal effects on selected commensal and opportunistic microorganisms that constitute the gut microbiota. Given the widespread use of these agents in diagnostic imaging, understanding their potential impact on microbial viability is of clinical importance.

Material and Methods: Strains of *Candida* spp., *Lactobacillus* spp., *Staphylococcus epidermidis*, *Enterococcus* spp., and *Escherichia coli* were cultured under standard laboratory conditions. Each strain was exposed to five different iodinated contrast agents at five dilution levels (1/1, 1/2, 1/4, 1/8, and 1/16). Antimicrobial activity was assessed using the disk diffusion method, a widely accepted in vitro technique for evaluating the inhibitory effects of chemical agents on microbial growth. All cultures were incubated aerobically at 37°C for periods ranging from 18 to 48 hours, depending on the growth characteristics of each microorganism.

Results: None of the tested contrast agents showed detectable bacteriostatic or bactericidal effects against any of the microorganisms at any dilution level. Growth patterns of all strains were consistent across different agent types and concentrations, indicating no inhibition of microbial proliferation.

Conclusion: Under controlled in vitro conditions, the iodinated contrast agents tested—including Ultravist 370, Omnipaque 350, Biemexol 350, Kopaq 300, and Omnipol 350—did not demonstrate any antimicrobial activity against selected gut microorganisms. These findings suggest that these agents are unlikely to directly affect gut microbial viability in the short term, supporting their safety in terms of maintaining normal intestinal microbial populations during routine diagnostic procedures.

Keywords: Iodinated Contrast Agents; Gut Microbiota; Antimicrobial Effects; Disk Diffusion

ÖZET

Amaç: Bu çalışmanın amacı, yaygın olarak kullanılan iyotlu kontrast ajanlarının, bağırsak mikrobiyotasını oluşturan seçilmiş kommensal ve fırsatçı mikroorganizmalar üzerinde bakteriyostatik veya bakterisidal etkiler gösterip göstermediğini araştırmaktır. Bu ajanların tanısal görüntülemede yaygın olarak kullanılması göz önüne alındığında, mikroorganizma canlılığı üzerindeki olası etkilerini anlamak klinik açıdan önem taşımaktadır.

Gereç ve Yöntemler: *Candida* spp., *Lactobacillus* spp., *Staphylococcus epidermidis*, *Enterococcus* spp. ve *Escherichia coli* suşları standart laboratuvar koşullarında kültüre edildi. Her suş, beş farklı iyotlu kontrast ajana beş farklı seyreltme düzeyinde (1/1, 1/2, 1/4, 1/8 ve 1/16) maruz bırakıldı. Antimikrobiyal etkinlik, kimyasal ajanların mikroorganizma büyümesini inhibe etme etkilerini değerlendirmek için yaygın olarak kullanılan in vitro bir yöntem olan disk difüzyon yöntemi ile değerlendirildi. Tüm kültürler, mikroorganizmaların büyüme özelliklerine bağlı olarak 18–48 saat süreyle 37°C’de aerobik koşullarda inkübe edildi.

Bulgular: Test edilen kontrast ajanlarının hiçbirisi, herhangi bir mikroorganizma üzerinde herhangi bir seyreltme düzeyinde bakteriyostatik veya bakterisidal etki göstermedi. Tüm suşların büyüme paternleri, ajan türü ve konsantrasyonundan bağımsız olarak tutarlıydı ve mikroorganizma çoğalmasının engellenmediğini gösterdi.

Sonuç: Kontrollü in vitro koşullarda, Ultravist 370, Omnipaque 350, Biemexol 350, Kopaq 300 ve Omnipol 350 dahil olmak üzere test edilen iyotlu kontrast ajanlar, seçilmiş bağırsak mikroorganizmaları üzerinde antimikrobiyal aktivite göstermedi. Bu bulgular, bu ajanların kısa vadede bağırsak mikrobiyal canlılığını doğrudan etkilemesinin olası olmadığını ve rutin tanısal işlemler sırasında normal bağırsak mikrobiyal popülasyonlarının korunması açısından güvenli olduklarını düşündürmektedir.

Anahtar Kelimeler: İyotlu Kontrast Ajanlar; Bağırsak Mikrobiyotası; Antimikrobiyal Etkiler; Disk Difüzyon

INTRODUCTION

In modern medicine, medical imaging is indispensable for diagnosing and monitoring diseases (1). Imaging techniques such as computed tomography (CT), magnetic resonance imaging (MRI), and ultrasonography facilitate non-invasive evaluation of internal organs and early detection of pathological changes. To enhance the effectiveness of these techniques, contrast agents are frequently used to more clearly visualize tissue density differences, and therefore, lesions (2). These agents are critical for comprehensively evaluating vascular structures, the gastrointestinal system, and hollow organs (2, 3). Contrast agents can be administered through various routes, such as oral, rectal, and intravenous, depending on the clinical indication (3). Oral or rectal contrast agents are preferred for gastrointestinal imaging, while intravenous agents are used for detailed evaluations of the circulatory system and internal organs (3). The route of administration directly affects both image quality and systemic absorption, potentially influencing biological effects.

Iodinated contrast agents are the most frequently used in radiological imaging, especially CT. Iodine, with its high atomic number, strongly absorbs X-rays, creating contrast. Non-ionic, low-osmolar formulations are preferred in clinical practice because of their good biological tolerance and low toxicity (3). While these agents are primarily excreted renally, a portion of them can be eliminated through the gastrointestinal system. This raises concerns about their potential impact on a person's gut microbiota, which refers to the community of microorganisms living symbiotically within their body.

The compositions of a person's microbiotas are influenced by numerous factors such as genetics, birth method, diet, environmental exposures, and medication use. These microbiotas densely populate different regions of the body, including the gastrointestinal tract, skin, respiratory tract, oral cavity, and urogenital system, and they play vital roles in maintaining the host's health (4, 5).

The gut microbiota is the most densely populated microbial ecosystem in the human body, consisting of trillions of microorganisms—mainly Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria—

that contribute to fermentation, short-chain fatty acid production, immune regulation, and maintenance of intestinal barrier integrity (6–8).

Disruptions in this ecosystem, known as dysbiosis, are linked to several conditions including, irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), obesity, diabetes, neuropsychiatric disorders, and cancer, largely through impaired immunity and chronic inflammation (6,9). The microbiota is highly sensitive to external factors such as antibiotics, diet, medications, and chemical exposures (10).

In this context, the impact of iodinated contrast agents on gut microorganisms remains poorly understood (11,12). Although early ionic agents containing free iodine exhibited antimicrobial activity, evidence regarding modern non-ionic agents is limited. Mosler et al. suggested that residual free iodine may provide minimal antimicrobial effects (13). These findings highlight the need to investigate the potential direct or indirect influence of iodinated contrast agents on gut microbiota.

MATERIALS AND METHODS

This study was approved by the Sivas Cumhuriyet University Ethics Committee (Decision No: 2025/07-07). Five different microorganisms were included in the study: *Candida* spp., *Lactobacillus* spp., coagulase-negative *Staphylococcus epidermidis*, *Enterococcus* spp., and *Escherichia coli*. All strains were American Type Culture Collection standard strains and were stored in milk at -20°C until the study day. One day before the study, they were subcultured onto appropriate media and revitalized.

The contrast agents tested were Ultravist 370 mg/ml, Omnipaque 350 mg/ml, Biemexol 350 mg/ml, Kopaq 300 mg/ml, and Omnipol 350 mg/ml. For each contrast agent, solutions were prepared immediately before use with distilled water at dilution ratios of 1/1 (undiluted), 1/2, 1/4, 1/8, and 1/16.

The disk diffusion method was used to detect and measure the antimicrobial activities of radioopaque substances. Whatman paper (Whatman No.4) was punched with a hole punch, and the obtained paper disks were placed in glass petri dishes and sterilized in an autoclave at 121°C for 15 minutes. Each disk was impregnated with 20 µl of contrast agent solution.

Following EUCAST (The European Committee on Antimicrobial Susceptibility Testing) recommendations, coagulase-negative *Staphylococcus epidermidis*, *Enterococcus* spp., *Lactobacillus*, and *Escherichia coli* strains were prepared to 0.5 McFarland, and *Candida* spp. to 1 McFarland in IDBroth. From the prepared solutions, *Staphylococcus epidermidis*, *Escherichia coli*, and *Candida* spp. were inoculated onto Mueller-Hinton agar (MHA) using swab sticks for lawn plating. *Enterococcus* spp. and *Lactobacillus* were lawn-plated onto blood agar media. Within five minutes, contrast agent-impregnated Whatman papers were placed onto the media using forceps.

Plates with disks were placed in an incubator at 35±1°C and incubated for 18±2 hours. Evaluations were performed by measuring with a ruler from the back of the plate against a dark background for MHA, and from the front with the plate lid open for disks placed on blood agar media. Due to the qualitative nature of growth observations (presence or absence of growth) and the lack of quantitative measurements, formal statistical analysis was not performed. All disk diffusion assays were performed in triplicate to ensure

reproducibility.

RESULTS

All tested microorganisms showed visible growth at all dilution levels of each contrast agent (Table 1, Figure 1 a-b). No bacteriostatic (growth inhibition) or bactericidal (growth cessation) effects were observed in any group. All microorganisms *Lactobacillus* spp., *Enterococcus* spp., *Staphylococcus epidermidis*, *Escherichia coli*, and *Candida* spp. successfully grew in the presence of Ultravist 370, Omnipaque 350, Biemexol 350, Kopaq 300, and Omnipol 350 at 1/1, 1/2, 1/4, 1/8, and 1/16 dilutions.

No observable differences in growth patterns were detected between different contrast agents or between various dilution levels (Table 2). No inhibition zone formation was detected in any contrast agent in the disk diffusion test (Figure 1 a-b). All microorganisms showed normal growth patterns around contrast agent disks, and no growth restriction was observed. The diameter of inhibition zones remained the same as the disk diameter in all cases (6 mm), indicating no antimicrobial activity.

Table 1. Results of antimicrobial activity of iodinated contrast agents against tested microorganisms by disk diffusion method.

Microorganism	Ultravist 370	Omnipaque 350	Biemexol 350	Kopaq 300	Omnipol 350
	Dilution Levels (1/1, 1/2, 1/4, 1/8, 1/16)				
<i>Candida</i> spp.	(-), (-), (-), (-), (-)	(-), (-), (-), (-), (-)	(-), (-), (-), (-), (-)	(-), (-), (-), (-), (-)	(-), (-), (-), (-), (-)
<i>Lactobacillus</i> spp.	(-), (-), (-), (-), (-)	(-), (-), (-), (-), (-)	(-), (-), (-), (-), (-)	(-), (-), (-), (-), (-)	(-), (-), (-), (-), (-)
<i>S. epidermidis</i>	(-), (-), (-), (-), (-)	(-), (-), (-), (-), (-)	(-), (-), (-), (-), (-)	(-), (-), (-), (-), (-)	(-), (-), (-), (-), (-)
<i>Enterococcus</i> spp.	(-), (-), (-), (-), (-)	(-), (-), (-), (-), (-)	(-), (-), (-), (-), (-)	(-), (-), (-), (-), (-)	(-), (-), (-), (-), (-)
<i>E. coli</i>	(-), (-), (-), (-), (-)	(-), (-), (-), (-), (-)	(-), (-), (-), (-), (-)	(-), (-), (-), (-), (-)	(-), (-), (-), (-), (-)

(-) No inhibition zone/bacterial growth present. No antimicrobial effect was detected for any microorganism at all contrast agents and dilution levels.

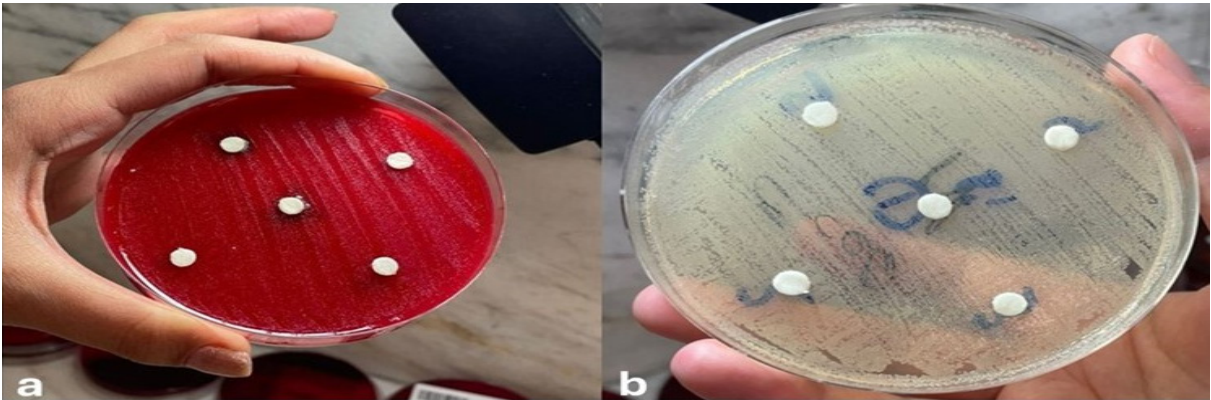


Figure 1. a. Application of disk diffusion method for measuring antibiotic resistance on blood agar medium.
b. Application of disk diffusion method for measuring antibiotic resistance on Mueller-Hinton agar medium.

Table 2. Microbial growth evaluation according to incubation periods.

Contrast Agent	Incubation Period	Microbial Growth Status
Ultravist 370	18 hours	Positive growth in all microorganisms
	24 hours	Positive growth in all microorganisms
	48 hours	Positive growth in all microorganisms
Omnipaque 350	18 hours	Positive growth in all microorganisms
	24 hours	Positive growth in all microorganisms
	48 hours	Positive growth in all microorganisms
Biemexol 350	18 hours	Positive growth in all microorganisms
	24 hours	Positive growth in all microorganisms
	48 hours	Positive growth in all microorganisms
Kopaq 300	18 hours	Positive growth in all microorganisms
	24 hours	Positive growth in all microorganisms
	48 hours	Positive growth in all microorganisms
Omnipol 350	18 hours	Positive growth in all microorganisms
	24 hours	Positive growth in all microorganisms
	48 hours	Positive growth in all microorganisms

DISCUSSION

In this in vitro study, none of the five iodinated contrast agents—Ultravist 370, Omnipaque 350, Biemexol 350, Kopaq 300, and Omnipol 350—demonstrated bactericidal or bacteriostatic activity against *Candida* spp., *Lactobacillus* spp., *Staphylococcus epidermidis*, *Enterococcus* spp., or *Escherichia coli*. These findings indicate that, under standard laboratory conditions, iodinated contrast agents do not directly inhibit microbial growth.

This study contributes to the limited literature evaluating the direct effects of modern non-ionic iodinated contrast agents on microorganisms relevant to the gut microbiota. Given their widespread use in diagnostic imaging, particularly during gastrointestinal examinations, understanding their microbiological safety is clinically important. Oral contrast agents are frequently used to improve visualization of bowel pathology, and therefore clarifying their interaction with gut microorganisms holds clinical relevance.

Although CT enterography (CTE) is now a key modality for small bowel imaging, especially in inflammatory bowel disease, the detailed technical aspects of contrast administration vary across clinical settings. Neutral oral contrast agents are preferred because they enhance assessment of bowel wall enhancement without obscuring mucosal detail. As patients typically

ingest relatively large volumes of oral contrast during CTE, evaluating whether these agents affect gut microbiota is of practical importance. Our findings provide reassurance that commonly used iodinated contrast agents do not exert direct antimicrobial effects on representative commensal or opportunistic microorganisms (14,15).

Previous studies have reported inconsistent antimicrobial properties among contrast agents. Brönnimann et al. found minimal inhibition at pH 7.4 and more notable suppression at acidic pH, suggesting that pH may modulate antimicrobial effects. However, no inhibition was observed against *S. aureus* or *P. aeruginosa*, consistent with our findings (16). Older ionic agents were known to possess antimicrobial effects due to free iodine content, whereas modern non-ionic formulations appear to have minimal or no direct activity (12,13). Our results further support the view that current iodinated contrast agents have limited antimicrobial potential.

The clinical implications of these findings are noteworthy. Since contrast-enhanced imaging is widely used across diverse patient populations, including those with inflammatory bowel disease or chronic conditions requiring repeated examinations, the absence of direct antimicrobial effects reduces concern about potential acute disruption of gut microbial populations.

This study has several limitations. The *in vitro* design does not reflect the complexity of the human gastrointestinal environment, where factors such as pH fluctuations, osmolarity, host immunity, microbial interactions, and metabolic activity all contribute to microbial dynamics (5,6). Our experiments were conducted under standard laboratory pH (7.0–7.4), and therefore do not account for potential pH-dependent effects described in previous literature. Additionally, anaerobic gut conditions could not be simulated, and many dominant gut microorganisms were not represented in our model.

While no direct antimicrobial activity was observed, indirect effects of contrast agents—such as alterations in intestinal permeability, osmotic balance, or metabolic signaling—cannot be excluded (8). Future studies using fecal microbiota cultures, gut-on-a-chip models, animal experiments, and metagenomic approaches are required to better elucidate contrast agent–microbiota interactions and to assess long-term or cumulative exposure effects.

CONCLUSION

In conclusion, this study demonstrated that iodinated contrast agents do not possess bactericidal or bacteriostatic effects under *in vitro* conditions. While these findings provide preliminary evidence suggesting that contrast-enhanced imaging procedures are safe in terms of microbial viability in the short term, they do not exclude the possibility of indirect or long-term effects on the gut microbiota. Advanced studies incorporating more comprehensive, physiologically realistic, and *in vivo* models are urgently needed to fully elucidate this topic.

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