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RESEARCH ARTICLE OPEN ACCESS

Phenotypic and molecular characterisation of efflux pump genes in clinical isolates of Serratia marcescens

Characterisation of Efflux Pumps in S. marcescens

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Abstract

Objective: This study focuses on identifying and understanding efflux pump genes in Serratia marcescens bacteria collected from clinical samples. We aimed to ascertain how these bacteria resist antibiotics by characterising their outward features and genetic makeup.

Methods: We examined twenty isolates of S. marcescens, from urinary tract infections, diarrheal cases, and blood infections. To identify these bacteria, we used standard laboratory tests. We then tested whether they had active efflux pumps with a simple dye-based method called the ethidium bromide-agar cartwheel test. After that, we extracted their DNA and used PCR to look for five specific genes linked to efflux activity: SdeXY-Y, SsmE, SdeCDE-D, SdeAB-B, and LUXR. We applied the usual PCR conditions with primers designed for each gene.

Results: Our results showed that nearly half of the isolates (8 out of 20) had active efflux pumps. The LUXR gene was found in all the bacteria, making it the most common. The SdeCDE-D gene was present in 95% of samples, SdeAB-B in 90%, SdeXY-Y in 60%, and SsmE in 45%. The presence of these genes varied depending on the type of sample, with the highest occurrence in blood and urinary tract infections. To review, we confirmed that multiple efflux pump genes are present in clinical S. marcescens isolates. Notably, LUXR, SdeCDE-D, and SdeAB-B are the most widespread.

Conclusion: These findings suggest that efflux pumps could play an important role in antibiotic resistance, emphasising the need for ongoing genetic monitoring for optimal management of multidrug-resistant infections.

Keywords: Serratia marcescens, Multidrug Resistance, Efflux Pumps, Polymerase Chain Reaction, Microbial Sensitivity Tests

Plain English Summary

Serratia marcescens is a type of bacteria that can cause serious infections, especially in hospital patients. These bacteria are becoming increasingly resistant to treatment because they can resist many antibiotics. One way they do this is by using "efflux pumps", proteins that help the bacteria push antibiotics out of their cells before the drugs can work. In this study, we collected samples from patients in two hospitals in Iraq and identified 20 S. marcescens bacteria. We tested them in the lab to see if they used efflux pumps and checked their DNA to find specific genes linked to these pumps. We found that eight of the 20 bacteria showed signs of active efflux pumps. However, many more had the genes needed to make them. One gene, called LUXR, was found in all the bacteria and may help them communicate with each other and increase their resistance. Other genes, SdeCDE-D, SdeAB-B, SdeXY-Y, and SsmE, were also common and are known to help bacteria resist different antibiotics.

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Although we studied only a small number of samples, our results suggest that S. marcescens in this region may harbour hidden resistance traits. This makes infections harder to treat and underscores the need for improved testing and monitoring of antibiotic resistance. Understanding these resistance genes may also help scientists develop new treatments that block the efflux pumps, making antibiotics more effective.

Introduction

Serratia marcescens is a Gram-negative, rod-shaped bacterium characterised by motility but lacking the ability to form spores. It belongs to the order Enterobacterales and is a member of the Enterobacteriaceae family (1). Recognised broadly as an opportunistic pathogen, it is known to cause a diverse array of infections acquired within healthcare settings. Although it was once regarded as a non-virulent organism, recent evidence has positioned S. marcescens as a clinically important pathogen involved in conditions such as pneumonia, urinary tract infections, bacteraemia, endocarditis, osteomyelitis, and infections associated with intravenous catheters (2, 3).

The ability of S. marcescens to persist in hospital environments is partly attributed to its remarkable adaptability and capacity to produce enzymes like DNases, enhancing its resilience in these settings (4). More critically, the bacterium exhibits resistance to multiple drugs, primarily driven by various mechanisms, including the active efflux of antibiotics through specialised transporter proteins.

Efflux pumps are transmembrane proteins responsible for removing toxic compounds, such as antibiotics, detergents, dyes, heavy metals, and antimicrobial molecules produced by the host, from bacterial cells (5, 6). These systems not only contribute to antibiotic resistance across multiple classes but also play integral roles in bacterial physiology, influencing processes like quorum sensing, biofilm development, and pathogenicity (7, 8). Their activity greatly enhances both innate and acquired antimicrobial resistance in clinical strains (9, 10).

Several efflux systems belonging to the resistance-nodulation-cell division (RND) family and the small multidrug resistance (SMR) family have been identified within S. marcescens. Prominent among these are the SdeXY, SdeCDE, and SdeAB systems, each associated with resistance to antibiotics such as fluoroquinolones, tetracyclines, and β-lactams (11, 12, 13, 14). Also, the SsmE gene encodes an SMR-type efflux pump linked to resistance against norfloxacin and ethidium bromide (Minato et al., 2008). Though not a classical efflux gene, LUXR functions as a regulator of quorum sensing and may indirectly influence efflux pump expression (15, 16, 17). Recent reviews (7, 18) have emphasised the growing clinical importance of efflux-mediated resistance, emphasising the urgent need for molecular surveillance of these mechanisms in hospital isolates.

In this context, the present study aimed to evaluate both the phenotypic presence and the molecular distribution of specific efflux pump-related genes, namely SdeXY-Y, SsmE, SdeCDE-D, SdeAB-B, and LUXR, in S. marcescens isolates obtained from patients with various infections in Diwaniyah, Iraq. These genes were thoughtfully selected based on their documented roles in multidrug resistance and their clinical significance in S. marcescens, supported by recent molecular and functional research (4, 19, 20).

Materials and Methods

Study Design

This study employed a cross-sectional, laboratory-based design and was carried out over the period from July 29 to November 26, 2024. Clinical samples were obtained from patients treated at Diwaniyah Teaching Hospital and the Women's and Children's Hospital, both located within Diwaniyah Governorate, Iraq.

Sample Collection and Bacterial Isolation

In total, 200 clinical specimens were collected from individuals presenting with urinary tract infections (n=91), diarrhoea (n=62), and bloodstream infections (n=47). These samples were cultured on selective media, and colonies exhibiting morphological features consistent with Serratia marcescens were subjected to standard biochemical identification procedures. A total of twenty isolates were confirmed as S. marcescens and included in the study, with isolates selected consecutively until the target sample size was reached.

Phenotypic Detection of Efflux Pump Activity

Phenotypic assessment of efflux pump activity was conducted using the ethidium bromide-agar cartwheel method as described by Martins et al. (2011). Bacterial suspensions were adjusted to a turbidity of 0.5 McFarland and streaked radially onto tryptic soy agar plates containing different concentrations of ethidium bromide (0.5, 1.0, 1.5, and 2.0 µg/mL). Following incubation at 37°C for 18–24 hours, plates were examined under UV light. A positive outcome, indicating active efflux, was identified when bacterial colonies showed weak or no fluorescence, signifying ethidium bromide extrusion. Conversely, intense

fluorescence was interpreted as a negative result for efflux pump function.

Genomic DNA Extraction

Genomic DNA was extracted from overnight cultures of S. marcescens using a commercial bacterial DNA extraction kit (ABM®, Canada), following the manufacturer's instructions. DNA purity and concentration were quantified using a NanoDrop™ spectrophotometer at 260/280 nm. Extracted DNA samples were stored at −20°C until further analysis.

Polymerase Chain Reaction (PCR) for Efflux Pump Gene Detection

Polymerase chain reaction (PCR) assays were performed to detect five genes associated with efflux pump activity: SdeXY-Y, SsmE, SdeCDE-D, SdeAB-B, and LUXR. The universal 16S rRNA gene served as a marker for species confirmation. Primer sequences and PCR cycling parameters are detailed in Table 1. Each 25 µL reaction mixture included 2.5 µL of 10× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM of each primer, 1 U of Taq DNA polymerase (Thermo Scientific®), and 2 µL of template DNA. Amplification was carried out using a thermal cycler (Bio-Rad®). All PCR runs incorporated positive controls, comprising previously characterised S. marcescens strains harbouring the target genes, and negative controls lacking DNA. Amplified products were separated on 1.5% agarose gels stained with ethidium bromide, then visualised under UV transillumination.

Table 1. Primer sequences and PCR cycling conditions for target genes

Gene	Primer Sequence (5'–3')	Product Size (bp)	Annealing Temp (°C)	Cycles
SdeXY-Y	F: CAGTGGTCGCTTCAACATTC R: GCTTACCTGGTGCTTCTTCC	488	58	35
SsmE	F: ATGACCGCGTTGTCATCATT R: TTAGGCGTTCGAGGATGTTG	188	55	35
SdeCDE-D	F: CGTTGACGACATTGACGAGT R: ACGAGGATGGTTTCGTCACT	417	56	35
SdeAB-B	F: AGCAGCGTATCGACCTTGTA R: CTGGTTGATCTGCGTCTTTC	415	57	35
LUXR	F: AAGCGCTTGCTGTGTTTGTA R: CTGCTGATCGAGGAAGTTCA	208	54	35
16S rRNA	F: AGAGTTTGATCCTGGCTCAG R: TACGGTTACCTTGTTACGACTT	1500	55	35

Data Analysis

Data regarding the prevalence of efflux pump genes were expressed as percentages. Descriptive statistical methods summarise both phenotypic and genotypic data. Due to the limited sample size, inferential statistical testing was not performed; nonetheless, qualitative descriptions of observed trends were included.

Results

Phenotypic Detection of Efflux Pump Activity

Out of the 20 Serratia marcescens clinical isolates tested, 8 (40%) demonstrated positive efflux activity using the ethidium bromide-agar cartwheel method (EtBr-CW). Fluorescence intensity under ultraviolet light was used to differentiate efflux-positive (weak or no fluorescence) from efflux-negative (strong fluorescence) isolates. The distribution of fluorescence at different EtBr concentrations is summarised in Table 2.

Table 2: Fluorescence pattern of S. marcescens isolates on EtBr-containing agar plates (interpreted for efflux pump activity)

Isolate ID	0.5 µg/mL	1.0 µg/mL	1.5 µg/mL	2.0 µg/mL	Efflux Pump Activity
Sm1	–	–	–	+	Negative
Sm2	+	+	–	–	Positive
Sm3	–	–	–	–	Negative
Sm4	–	–	–	–	Negative
Sm5	–	–	–	–	Negative
Sm6	–	+	+	–	Positive
Sm7	+	–	–	–	Positive
Sm8	+	+	–	–	Positive

Sm9	–	–	–	–	Negative
Sm10	+	–	–	+	Positive
Sm11	+	+	+	+	Positive
Sm12	+	+	–	–	Positive
Sm13–Sm20	–	–	–	–	Negative

(+ = Fluorescent; – = No fluorescence; ND = Not detected)

Representative examples of fluorescence differential staining at various EtBr isolates are shown in Figure 1, demonstrating concentrations.

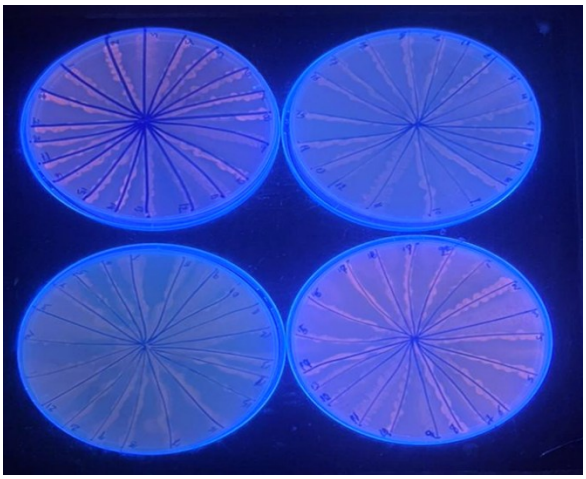

Figure 1: Fluorescent responses of S. marcescens isolates stained with ethidium bromide (0.5–2.0 µg/mL) under UV light. Positive efflux activity was indicated by reduced fluorescence intensity

Molecular Detection of Efflux Pump and Quorum Sensing Genes

PCR analysis confirmed the presence of five target genes associated with efflux activity and quorum sensing. The distribution of these genes among the 20 S. marcescens isolates is summarised in Table 3.

Table 3: Frequency of efflux and quorum-sensing genes detected by PCR

Gene	No. of Positive Isolates	Prevalence (%)
LUXR	20	100%
SdeCDE-D	19	95%
SdeAB-B	18	90%
SdeXY-Y	12	60%
SsmE	9	45%

Amplification of gene products was confirmed by agarose gel electrophoresis. Representative gel images for each gene are shown in Figures 2 to 6.

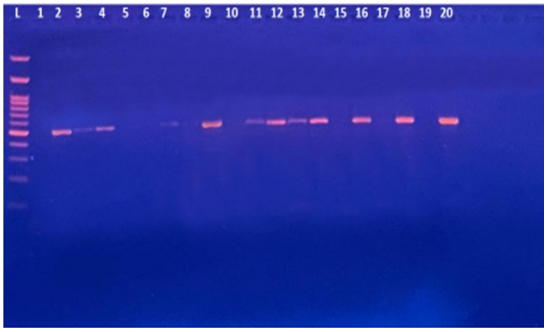

Figure 2: PCR amplification of SdeXY-Y gene (488 bp) in S. marcescens isolates

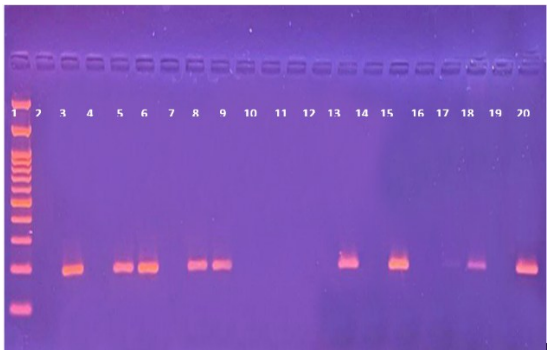

Figure 3: PCR amplification of SsmE gene (188 bp)

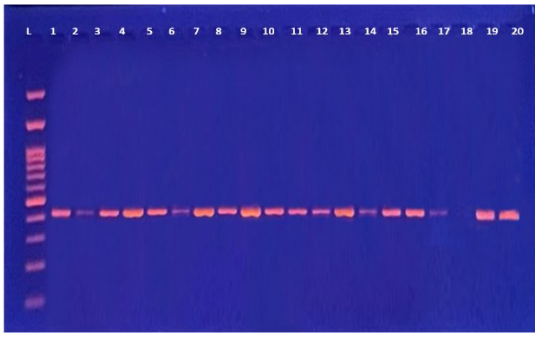

Figure 4. PCR amplification of SdeCDE-D gene (417 bp)

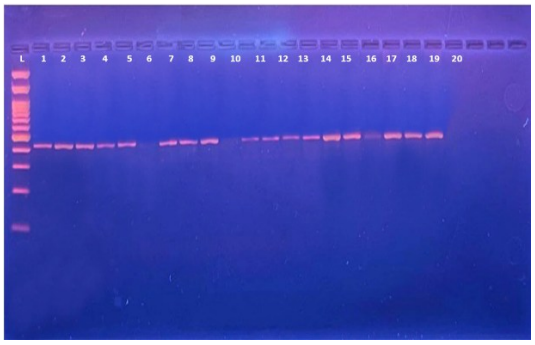

Figure 5. PCR amplification of SdeAB-B gene (415 bp)

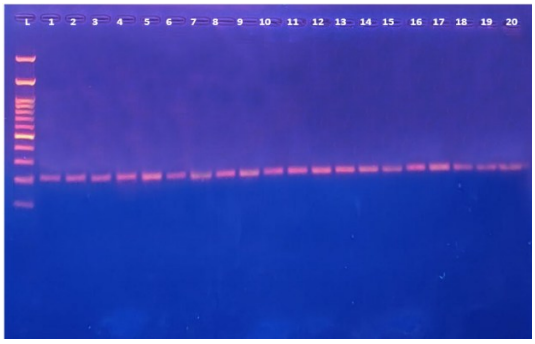

Figure 6. PCR amplification of the LUXR gene (208 bp)

Each PCR reaction included appropriate positive and negative controls. Gene-specific bands were observed at expected product sizes, confirming successful amplification. Isolate-wise presence/absence for each gene is detailed in Table 4.

Table 4: Presence (+) or Absence (–) of Efflux Pump and Quorum Sensing Genes in Serratia marcescens Isolates

Isolate ID	SdeXY-Y	SsmE	SdeCDE-D	SdeAB-B	LUXR
Sm1	–	–	+	+	+
Sm2	+	–	+	+	+
Sm3	–	–	+	+	+
Sm4	–	+	+	+	+
Sm5	+	–	+	+	+
Sm6	+	+	+	+	+
Sm7	–	–	+	–	+
Sm8	+	+	+	+	+
Sm9	+	–	+	+	+
Sm10	–	+	+	+	+
Sm11	+	+	+	+	+
Sm12	+	–	+	+	+
Sm13	–	–	–	–	+
Sm14	+	–	+	+	+
Sm15	–	–	+	+	+
Sm16	+	+	+	+	+
Sm17	–	–	+	+	+
Sm18	–	+	+	+	+
Sm19	+	+	+	+	+
Sm20	+	–	+	+	+

+ = Gene detected by PCR; – = Gene not detected; Sm1–Sm20 = S. marcescens isolates 1 through 20

Discussion

This investigation focused on the detection and distribution of key efflux pump and quorum sensing genes within Serratia marcescens isolates derived from clinical specimens in Diwaniyah, Iraq. Employing both phenotypic and molecular methodologies, we observed that approximately 40% of the isolates demonstrated phenotypic efflux activity, while a majority harboured multiple genes associated with efflux mechanisms. These findings emphasise the important role of efflux systems in shaping the antimicrobial resistance profile of S. marcescens, corroborating previous research (7, 8, 10).

A particularly noteworthy aspect of our study is the observed discrepancy between phenotypic results and genotypic data. Despite the widespread presence of efflux pump genes such as SdeXY-Y, SsmE, SdeCDE-D, and SdeAB-B, only 40% of the isolates exhibited phenotypic efflux activity when tested with the ethidium bromide cartwheel method. This inconsistency may be attributable to various factors, including differences in gene expression, mutations rendering genes non-functional, regulatory controls, or gene silencing during in vitro experimentation (12, 21). Efflux activity is often modulated by environmental stimuli, stress responses, and quorum sensing signals, conditions that might not have been fully replicated in the experimental setup.

Interestingly, the LUXR gene was detected in all 20 isolates (100%), which aligns with the well-established conserved function of LUXR homologues in quorum sensing among many Gram-negative bacteria, including S. marcescens (15, 17). These regulator genes can influence biofilm development, pathogenicity, and even efflux pump expression, hinting at a possible connection between quorum sensing and antimicrobial resistance mechanisms (16). Their universal presence emphasises the importance of LUXR in maintaining bacterial adaptability, particularly in clinical environments.

The RND-type efflux pump genes SdeCDE-D and SdeAB-B were found in 95% and 90% of isolates, respectively. These genes encode multidrug efflux systems capable of removing a broad scope of antibiotics, including β-lactams, fluoroquinolones, tetracyclines, and chloramphenicol (13, 22, 23, 24). Their high prevalence confirms previous observations from Iraq and other regions (4, 14), emphasising the clinical significance of efflux-based resistance.

The SdeXY-Y gene was identified in 60% of the isolates. This efflux system has been associated with resistance to tigecycline, erythromycin, novobiocin, and ciprofloxacin (11, 12, 13).

Variations in the presence of the SdeXY-Y gene among isolates may reflect selective pressures stemming from antimicrobial usage or local clonal dissemination. Notably, 45% of isolates carried the SsmE gene, which encodes an SMR-type efflux pump involved in resistance to norfloxacin and ethidium bromide (25). Although less common, the role of SsmE may become more prominent under particular environmental or antimicrobial conditions.

From a clinical standpoint, the presence of multiple efflux pump genes indicates that infections caused by S. marcescens in this environment may pose challenges to treatment with standard antibiotics. These efflux mechanisms are known to limit the intracellular concentration of drugs such as fluoroquinolones, tetracyclines, aminoglycosides, and chloramphenicol, potentially leading to treatment failures (7, 9). Besides, the simultaneous presence of quorum-sensing regulators like LUXR alongside efflux genes raises concerns about enhanced biofilm formation and the organism's ability to persist in cases of catheter-associated or chronic infections.

The detection of efflux-related genes across isolates from diverse infection sites, including urine, blood, and stool, emphasises the organism's remarkable adaptability and the necessity for strong antimicrobial stewardship and infection control measures. Clinical microbiology laboratories should consider incorporating fast molecular diagnostics for efflux genes into routine resistance profiling, especially in tertiary hospitals where S. marcescens is increasingly recognised as an important pathogen.

Study limitations

This investigation is subject to several limitations. Primarily, the relatively small sample size (n = 20) constrains the broader applicability of the findings and restricts the use of inferential statistical analyses. Besides, the study was geographically confined to a single location, Diwaniyah, Iraq, and thus may not represent the prevalence or genetic diversity of efflux mechanisms in other regions. The absence of antimicrobial susceptibility testing, including minimum inhibitory concentration (MIC) determinations, limits the ability to directly associate the presence of efflux genes with phenotypic resistance. Lastly, the study did not evaluate gene expression levels or the effects of efflux pump inhibitors, which would have offered deeper insight into their functional significance.

Going forward, larger, multicentre studies that integrate phenotypic resistance data with transcriptomic or proteomic analyses are essential. What's more, exploring the therapeutic potential of efflux pump inhibitors could open new avenues for combating resistant infections.

Conclusion

This investigation offers compelling evidence that clinical isolates of *Serratia marcescens* obtained from Diwaniyah, Iraq, carry various efflux pump genes, notably LUXR, SdeCDE-D, and SdeAB-B, which were detected most frequently. While phenotypic efflux activity was observed in only 40% of the isolates, the widespread presence of these efflux-related genes indicates a considerable potential for antimicrobial resistance, especially against β-lactams, fluoroquinolones, and tetracyclines. The consistent detection of the LUXR gene emphasises the probable involvement of quorum sensing in regulating efflux mechanisms and other virulence factors.

Despite these major insights, the study's scope was constrained by a limited sample size, the absence of comprehensive antibiotic susceptibility profiles, and its single-centre setting. These limitations emphasise the necessity for larger-scale, multi-institutional research that integrates molecular analyses, phenotypic assessments, and clinical outcome data. Future investigations should focus on evaluating the expression levels of these efflux genes under various antibiotic pressures and exploring the potential of efflux pump inhibitors as adjunct treatments.

From a public health and clinical standpoint, genes such as SdeCDE-D and regulatory elements like LUXR represent promising targets for resistance mitigation strategies. Strengthening surveillance efforts and incorporating molecular diagnostics into routine microbiology protocols could greatly enhance antimicrobial stewardship and infection control measures within hospital environments.

List of Abbreviations

Bp: Base pair
DNA: Deoxyribonucleic Acid
EtBr: Ethidium Bromide
EtBr-CW: Ethidium Bromide–Agar Cartwheel Method
IRB: Institutional Review Board
LUXR: LuxR-type quorum-sensing regulator gene
MIC: Minimum Inhibitory Concentration
MDR: Multidrug Resistance
PCR: Polymerase Chain Reaction
QS: Quorum Sensing
RND: Resistance-Nodulation-Division (efflux pump family)
RNA: Ribonucleic Acid
SMR: Small Multidrug Resistance (efflux pump family)

UTI: Urinary Tract Infection
UV: Ultraviolet

Declarations

Ethics approval and consent to participate

This study was conducted following the ethical principles outlined in the Declaration of Helsinki. Ethical approval was obtained from the Institutional Review Board (IRB) of the University of Al-Qadisiyah, Iraq (Approval Reference No.: [EDU-37-11/12/2024]). Before sample collection, written informed consent was obtained from all participants or, in the case of minors, from their parents or legal guardians. Confidentiality and anonymity of patient data were strictly maintained throughout the study.

Consent for Publication

All the authors gave consent for the publication of the work under the Creative Commons Attribution Non-Commercial 4.0 license.

Availability of Data

Data for this work is available from the authors and may be provided upon reasonable request.

Conflicts of Interest

None.

Funding

None.

Authors' contributions

KMM: Contributed to the procurement of samples, carried out part of the experimental work and statistical analysis, and was involved in writing and revising the manuscript.
HAN: Conceived of the research, led the design and implementation of the study, was involved in the data interpretation, and took responsibility for manuscript editing, review and final approval.

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