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Isolation of Multidrug-Resistant Bacteria (MDR) from Ruminant Skin Lesions in Dhaka City

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ABSTRACT

The occurrence of cutaneous infections in ruminants poses a significant threat to animal welfare and public health, particularly given the rise in antimicrobial resistance (AMR). In this study bacteria from skin wound infections of cattle, goats, and sheep were isolated and identified with their antibiogram profiles. A total of 100 clinical samples (cattle, n = 60; goats, n = 20; sheep, n = 20) were collected from various skin lesions of target animals in Dhaka, Bangladesh. Conventional cultural, morphological, and biochemical techniques were used to identify bacterial pathogens. Antimicrobial susceptibility testing was performed against nine commonly used antibiotics using the Kirby–Bauer disc diffusion method. *Staphylococcus* spp. was the most predominant isolate (51%), followed by *Bacillus* spp. (18%), *Dermatophilus* spp. (11%), and *Streptococcus* spp. (8%). Species-specific analysis revealed that *Staphylococcus* was most prevalent in cattle (53.33%), goats (50%), and sheep (45%). Maximum resistance was observed against β-lactam antibiotics. Conversely, the isolates demonstrated high susceptibility to fluoroquinolones (ciprofloxacin) and aminoglycosides (gentamicin). These findings underscore the high prevalence of multidrug-resistant (MDR) bacteria in ruminant skin wounds and emphasize the need for routine sensitivity testing to guide effective therapeutic interventions and combat the growing global health crisis of antimicrobial resistance.

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Introduction

Skin injuries in domestic animals - whether from trauma, bites, or surgical incisions—frequently lead to infections characterized by painful ulcerations, hair loss, and discharge (Tyler et al., 1989). While some bacteria, such as *Corynebacterium* and *Acinetobacter*, are harmless residents of normal skin, open wounds are highly susceptible to pathogenic contamination that can escalate from localized abscesses to systemic septicemia (Saini et al., 1992; Stulberg et al., 2002). Global research highlights *Staphylococcus aureus* and *Streptococcus pyogenes* as the primary culprits behind conditions such as folliculitis and cellulitis, alongside other significant pathogens, including *Pseudomonas aeruginosa*, *Bacillus anthracis*, and *Clostridium perfringens* (Swartz et al., 2000; Wilkerson et al., 2002). Specifically, in tropical regions, Dermatophilosis causes severe exudative dermatitis that can be fatal, while deeper wounds often harbor anaerobic pathogens like *Fusobacterium* and *Bacteroides*, or *E. coli* introduced via fecal contamination (Ali et al., 2003; Tiwari et al., 2015).

Historically, traditional livestock management often neglected these wounds until they compromised overall health, but the resulting skin diseases and parasite infestations now lead to significant economic losses by degrading the quality of hides, skins, and wool (Saini et al., 1992). This is particularly critical in Bangladesh, where the tanning industry produces 200 million square feet of leather annually from cows (56%), goats (33%), and buffalo (Paul et al., 2013). However, the improper use of allopathic medications without confirmed diagnoses, often characterized by underdosing or the use of broad-spectrum agents for minor lesions, has fuelled the rise of antimicrobial-resistant (AMR) microorganisms (Douglas et al., 1975; Okeke et al., 2005). As these pathogens evolve, standard treatments become ineffective, leading to chronic infections that permanently scar animal tissues and diminish leather value.

Despite the industry's scale, local research remains scarce; while international data are extensive, studies in Bangladesh have largely focused on general occurrence reports of ectoparasites or specific research on Dermatophilosis (Huq and Molla et al., 1972; Nooruddin and Dey et al., 1990). Consequently, there is an urgent need for comprehensive studies into the isolation and identification of diverse bacterial pathogens, coupled with rigorous antibiotic resistance profiling. Understanding the sensitivity patterns of these isolates is essential for developing targeted therapeutic protocols, reducing the spread of multidrug-resistant strains, and preventing the massive deterioration of the hide that threatens the national economy.

Materials and Methods

Study Area, Sample Collection, and Processing

The samples were collected from different areas of Dhaka city (Figure 1) during July 2024 to June 2025 and the whole experiment was conducted in the Department of Microbiology and Parasitology (MIPA), Sher-e-Bangla Agricultural University (SAU), Dhaka.

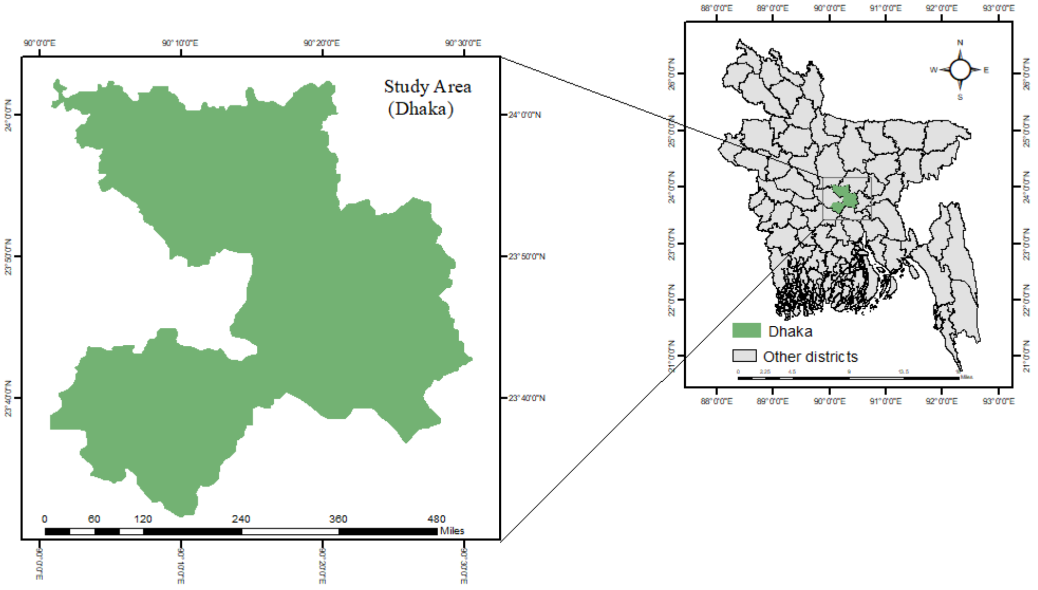


Figure 1. Map of Bangladesh highlighting the study area, Dhaka district, where clinical samples were collected from ruminants. The map was developed using ArcGIS (10.8).

A total of 100 clinical samples were collected from ruminants with various skin conditions, including cattle (n = 60), goats (n = 20), and sheep (n = 20). Sampling sites encompassed the SAU Veterinary Clinic (SAUVC), commercial dairy farms, and rural households within Dhaka city. Following visual clinical examination, specimens such as skin scrapings, crusts, swabs, and scabs were collected aseptically (Carter et al., 1993). All samples were placed in sterile test tubes and transported under aseptic conditions, maintaining the cold chain to the laboratory for further study.

Isolation and Identification of Bacterial Pathogens

Bacterial isolation was performed according to standard microbiological procedures (Martínez-Vázquez et al., 2021), with minor modifications. Initially, samples were enriched in 10 mL of nutrient broth (NB) (HiMedia, India) and incubated at 37°C for 24 hours. Presumptive growth was assessed via Gram's staining to determine morphological characteristics. To obtain pure cultures, the enriched broth was streaked onto nutrient agar (NA) and blood agar (BA) media. Further selective isolation was performed using mannitol salt (MS) agar (HiMedia, India) for Staphylococcus and Staphylococcus selection agar (HiMedia, India) for *Streptococcus*.

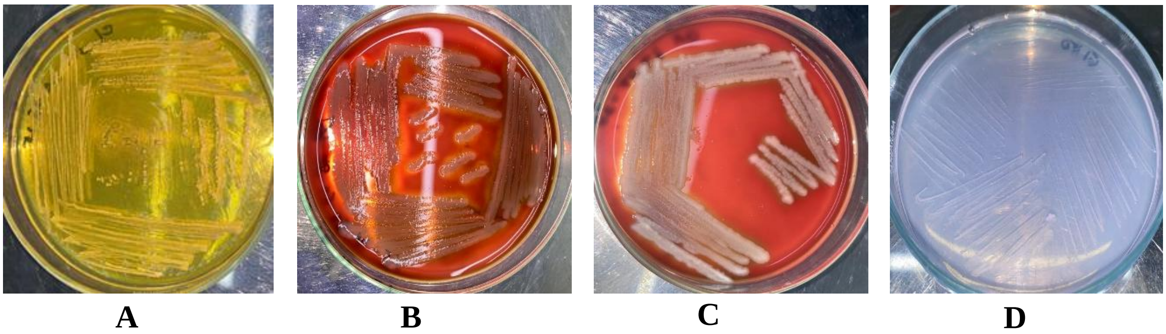
Antimicrobial Susceptibility Testing

The antibiogram profiles of the isolated pathogens were determined using the Kirby-Bauer disc diffusion method on mueller-hinton (MH) agar media, following CLSI (2024) guidelines. Fresh broth cultures were uniformly spread across the MH agar surface. Nine antimicrobial discs representing various classes were applied (HI Media, India): penicillins (ampicillin, 10 µg; penicillin G, 10 units), cephalosporins (cefotaxime, 30 µg), fluoroquinolones (ciprofloxacin, 5 µg), aminoglycosides (gentamicin, 10 µg), tetracyclines (tetracycline, 10 µg), macrolides (erythromycin, 15 µg), glycopeptides (vancomycin, 30 µg), and phenicols (chloramphenicol, 30 µg). After incubation at 37°C for 24 hours, the diameters of the zones of inhibition were measured and interpreted as sensitive (S), intermediate (I), or resistant (R). Multidrug resistance (MDR) was defined as resistance to more than two antimicrobial classes.

Results

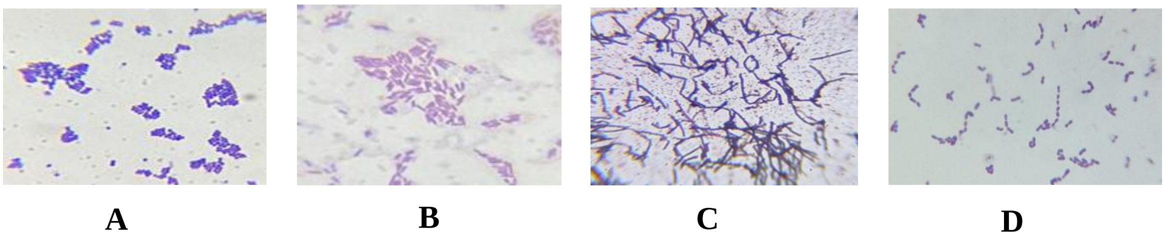
Cultural and Biochemical Confirmation of the Isolates

A total of 100 ruminant skin samples yielded diverse bacterial growth. *Staphylococcus* spp. were identified by their brilliant golden-yellow colonies on MS agar and confirmed as Gram-positive cocci in clusters. Biochemically, they were catalase- and MR-VP-positive, but indole-negative. *Bacillus* spp. produced white colonies on nutrient agar and hemolysis on blood agar, characterized as Gram-positive rods that were catalase and VP positive. *Dermatophilus* spp. were identified as Gram-positive branching filamentous bacteria producing yellowish colonies, while *Streptococcus* spp. formed small, whitish colonies on *Streptococcus* selection agar and appeared as Gram-positive cocci in chains (Figure 2; Figure 3).



A B C D

Figure 2. Cultural properties of the isolated bacteria. **A:** Brilliant golden yellow color colonies of *Staphylococcus* on MS agar, **B:** Beta hemolysis on blood agar produced by *Bacillus* spp., **C:** Hemolysis on blood agar by *Dermatophilus* spp., **D:** Smooth, small, whitish colonies of *Streptococcus* spp. on Streptococcus selection agar media.



A B C D

Figure 3: Results of Gram's staining, Gram positive round shaped cluster of *Staphylococcus* spp. (A), rod-shaped *Bacillus* spp. (B), branching filamentous *Dermatophilus* spp. (C), and round shaped chain of Stre*ptococcus* spp. (D).

Occurrence of Bacteria from Skin Samples

Among the 100 samples, the overall isolation rates of *Staphylococcus* spp. was 51%. Similarly, *Bacillus* spp. accounted for 18%, *Dermatophilus* spp. for 11%, and Streptococcus spp. for 8%, with 12% unidentified bacteria (non-specific) (Figure 4).

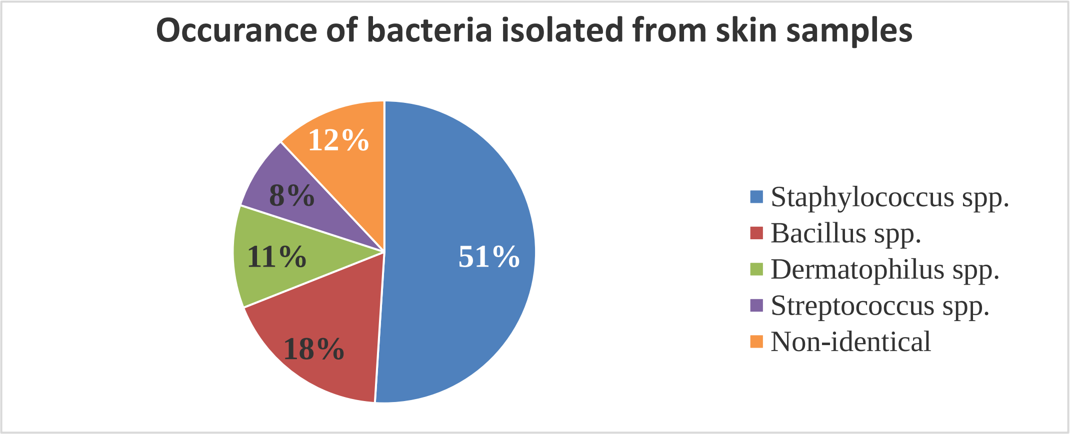


Figure 4. Percentage of bacterial occurrence in skin samples.

Species-wise distribution showed that *Staphylococcus* spp. was the most prevalent across all hosts, detected in 53.33% of cattle, 50% of goats, and 45% of sheep. *Bacillus* spp. and *Dermatophilus* spp. showed consistent occurrence across the three ruminant groups (Table 1).

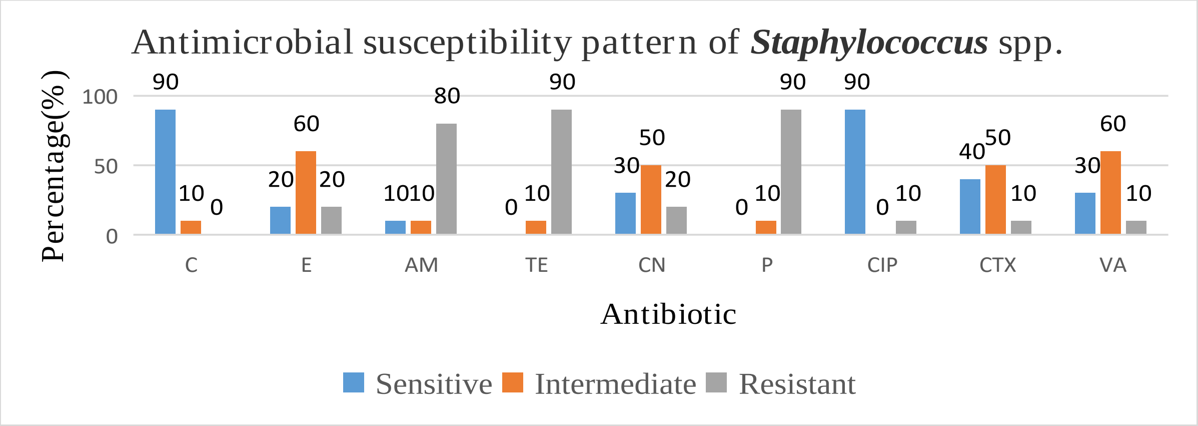
Table 1. Occurrence of isolated bacteria according to ruminant species

Ruminant Species	**No. of Sample (%)**	**Occurrence of bacterial species**				
		***Staphylococcus* spp.**	***Bacillus* spp.**	***Dermatophillus* spp.**	***Streptococcus* spp**	**UnIdentified**
Cattle	60 (60)	32 (53.33)	10 (16.67)	7 (11.67)	5 (8.33)	6 (10.0)
Goat	20 (20)	10 (50.0)	4 (20.0)	2 (10.0)	2 (10.0)	3 (15.0)
Sheep	20 (20)	9 (45.0)	4 (20.0)	2 (10.0)	1 (5.0)	3 (15.0)
Total	100 (100)	51 (51)	18 (18)	11 (11)	8 (8)	12 (12)

Antimicrobial Susceptibility Profiles of the Isolated Bacteria

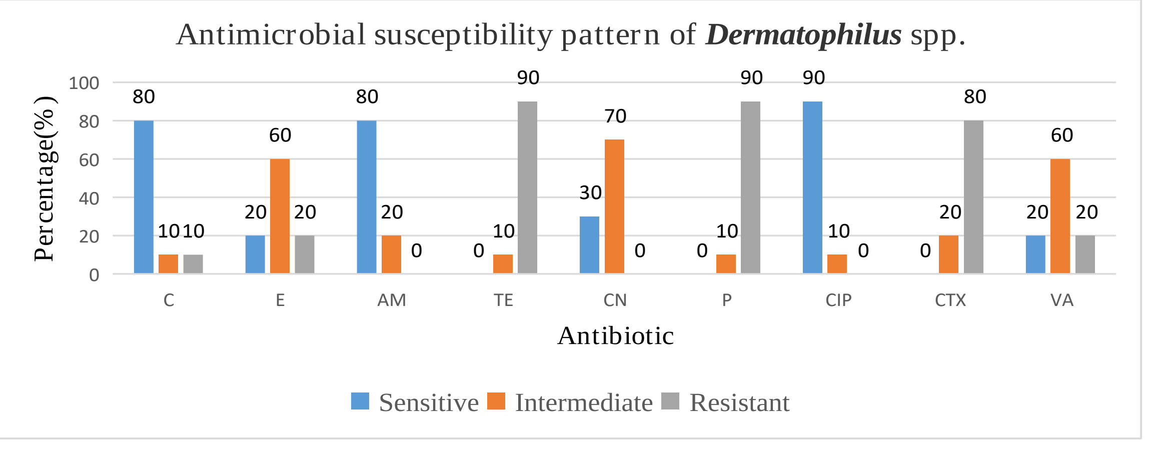
Antibiogram analysis revealed significant multidrug resistance (MDR) patterns across the isolated genera. *Staphylococcus* spp. exhibited high resistance to β-lactam, specifically penicillin (90%) and ampicillin (80%), as well as tetracycline (90%). Resistance was also noted against vancomycin (60%) and erythromycin (60%). Similarly, *Bacillus* spp. showed 90% resistance to tetracycline and 80% to both penicillin and ampicillin.

In contrast, high susceptibility was observed toward fluoroquinolones and aminoglycosides. *Staphylococcus* spp. and *Bacillus* spp. both showed 90% sensitivity to ciprofloxacin. *Streptococcus* spp. remained 80% sensitive to ciprofloxacin, cefotaxime, and ampicillin. Overall, the majority of isolates were classified as MDR, showing resistance to three or more antibiotic classes, particularly the β-lactam and tetracycline groups.



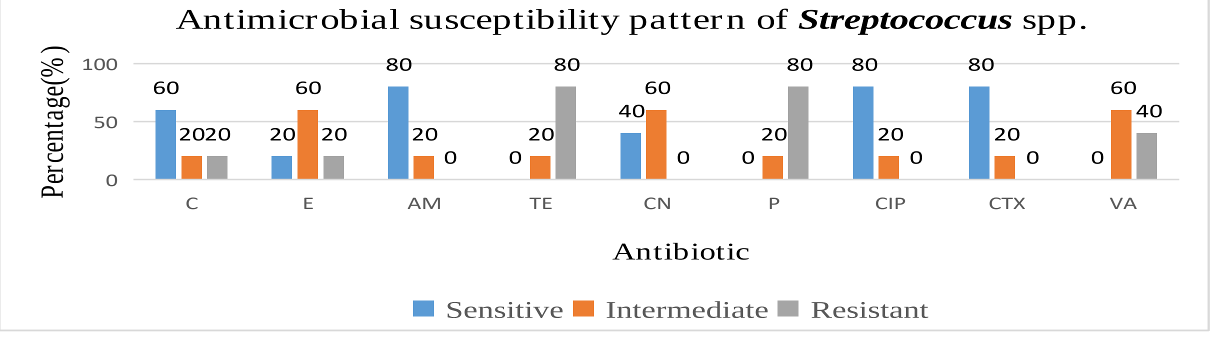
Legends: C= Chloramphenicol, E= Erythromycin, AM= Ampicillin, TE= Tetracycline, CN= Gentamicin, P= Penicillin G, CIP= Ciprofloxacin, CTX= Cefotaxime, VA= Vancomycin

Figure 5. Antimicrobial susceptibility pattern of *Bacillus* spp.



Legends: C= Chloramphenicol, E= Erythromycin, AM= Ampicillin, TE= Tetracycline, CN= Gentamicin, P= Penicillin G, CIP= Ciprofloxacin, CTX= Cefotaxime, VA= Vancomycin

Figure 6. Antimicrobial susceptibility pattern of *Dermatophilus* spp.



Legends: C= Chloramphenicol, E= Erythromycin, AM= Ampicillin, TE= Tetracycline, CN= Gentamicin, P= Penicillin G, CIP= Ciprofloxacin, CTX= Cefotaxime, VA= Vancomycin

Figure 7. Antimicrobial susceptibility pattern of *Streptococcus* spp.

Discussion

In this study, *Staphylococcus* spp. (51%) emerged as the predominant pathogen isolated from ruminant skin wounds. These findings are consistent with Staphylococci's role as common commensals that can transition into opportunistic pathogens following traumatic or surgical skin injury (Stulberg et al., 2002; Swartz et al., 2000). The high occurrence in cattle (53.33%) and goats (50%) aligns with regional observations in Bangladesh, where staphylococcal infections are the most frequently reported bacterial skin conditions (Nooruddin and Dey et al., 1990). The isolation of *Dermatophilus* spp. (11%) is of particular clinical significance, as it is the primary agent of bovine dermatophilosis. This condition not only affects animal health but also degrades hide quality, posing a threat to the economic value of the leather industry (Ali et al., 2003). The presence of *Bacillus* spp. (18%), often-considered environmental contaminants, suggests a high risk of secondary infection in open wounds under typical farm conditions (Tiwari et al., 2015).

The antimicrobial resistance (AMR) profiles observed are concerning, particularly the 80–90% resistance to penicillin and tetracycline. This mirrors trends seen in the Bangladeshi poultry sector, likely due to the widespread availability and unregulated use of these first-line drugs (Mehmood et al., 2020). The 60% resistance of Staphylococci to vancomycin, a drug of last resort in human medicine, highlights a critical One Health challenge, suggesting a reservoir of resistant genes that could potentially transition to the human population. Conversely, the high ciprofloxacin sensitivity (90%) across multiple genera suggests that fluoroquinolones remain a viable therapeutic option. However, as noted by Okeke et al. (2005), reliance on a limited pool of effective drugs increases selective pressure for the development of further resistance. The high Multidrug Resistance (MDR) rates observed in this study emphasize the urgent need for evidence-based prescribing and regular surveillance to mitigate the impact of resistant skin pathogens on both animal productivity and public health.

Conclusion

Staphylococcus, *Bacillus*, *Dermatophilus*, and *Streptococcus* were isolated and identified from 51%, 18%, 11%, and 8% of the skin samples, respectively. Since all four identified bacterial species are highly susceptible to ciprofloxacin and strongly resistant to ampicillin and Penicillin G, ciprofloxacin is an effective treatment for ruminant skin problems. To reduce risks to public health, antimicrobial drugs should not be administered carelessly, and proper hygiene procedures should be followed when handling animals with skin illnesses.

Conflict of interest

The authors showed no conflict of interest.

Acknowledgement

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Author's contribution

MKH: Data curation, formal analysis, investigation, methodology, and writing original draft. funding acquisition, resources, and supervision, MI: Formal analysis, writing – original draft, and writing – review & editing, MPS: Investigation, methodology, and writing – review & editing, MH: Investigation, methodology, and writing – review & editing, TA: Formal analysis, writing – original draft, and writing – review & editing, ABS: Formal analysis, and writing.

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