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# Current state and future directions for veterinary antimicrobial resistance research

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**ABSTRACT**

Antimicrobial resistance (AMR) is a critical One Health concern with implications for human, animal, plant, and environmental health. Antimicrobial susceptibility testing (AST), antimicrobial resistance testing (ART), and surveillance practices must be harmonized across One Health sectors to ensure consistent detection and reporting practices. Veterinary diagnostic laboratory stewardship, clinical outcomes studies, and training for current and future generations of veterinarians and laboratorians are necessary to minimize the spread of AMR and move veterinary medicine forward into an age of better antimicrobial use practices. The purpose of this article is to describe current knowledge gaps present in the literature surrounding ART, AST, and clinical or surveillance applications of these methods and to suggest areas where AMR research can fill these knowledge gaps. The related Currents in One Health by Maddock et al, *JAVMA*, March 2024, addresses current limitations to the use of genotypic ART methods in clinical veterinary practice.

**Keywords:** One Health, veterinary microbiology, antimicrobial susceptibility testing, laboratory stewardship, antimicrobial resistance

## Background

Antimicrobial resistance (AMR) is a One Health challenge with far-reaching implications for human, animal, plant, and environmental health.[1] In 2019, 1.27 million human deaths were attributed to bacterial AMR, and the impact is expected to continue to grow if substantive changes or interventions are not made across all health sectors.[2] The American Society for Microbiology and National Action Plan for Combating Antibiotic-Resistant Bacteria (2020–2025) advocate for a One Health approach to AMR, particularly in regard to research funding, to improve diagnostic methods, prevention strategies, and public health surveillance of AMR.[3,4]

Veterinary diagnostic laboratories (VDLs) are critical One Health partners in combatting AMR through the identification of pathogenic bacteria, through the performance of antimicrobial susceptibility testing (AST), and as a source of expertise for veterinarians regarding resistance mechanisms and therapeutics. VDLs are also critical in the communication of AMR data to human and animal public health practitioners. To maintain capacity and minimize the spread of AMR, diagnostic veterinary medicine requires resources, education, and advocacy to support appropriate antimicrobial use (AMU) and characterization of AMR challenges in animals. In this review, we will highlight areas of research

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and diagnostic support needed for standardization of laboratory testing, surveillance across the One Health spectrum, laboratory stewardship, clinical outcomes, and true One Health partnership. We will also suggest areas of opportunity for interdisciplinary collaboration as well as training and education needs for veterinarians and laboratorians.

## One Health Harmonization

AMR-centric studies with the greatest impact will consider the problem through an interdisciplinary lens **(Figure 1)**. The battle against AMR requires a robust, interdisciplinary approach, but the current system lacks harmonization in many areas. AMR phenotypes and genotypes of One Health concern are commonly discussed, but there is little consensus across the One Health spectrum regarding detection, reporting, and action based on the detection of AMR.[5–7] Nosocomial outbreaks with antimicrobial-resistant bacteria are predicted to become more common in veterinary medicine; these bacteria have zoonotic potential and reporting should be clear and unified.[5,8,9] For example, extensive AMR surveillance systems are in place for human medicine at the state level in the US, but often it is unclear whether an animal with a resistant infection of One Health concern, such as a carbapenemase-producing carbapenem-resistant Enterobacterales (CP-CRE), should be reported to state public health officials or if reporting is at the discretion of the respective Board of Animal Health or state animal health regulators.[5–9]

Additionally, there are no One Health-oriented criteria for classifying a bacterium tested as resistant to an antimicrobial or what constitutes a multidrug-resistant organism (MDRO). Several authors[10,11] have independently proposed criteria, but lack of consensus results in inconsistencies in what is resistant and ultimately defines an MDRO classification, leading to differences in perceived rates of resistance to multiple drugs between human and veterinary medicine. For example, a veterinary clinical breakpoint may call a bacterium resistant whereas the human breakpoint would call it susceptible due to differences in pharmacokinetics entirely unrelated to whether the bacterium does or does not possess an expressed resistance mechanism. To complicate matters further, the epidemiologic cut-off value, a better measure of acquired resistance, may not align with either veterinary or human clinical breakpoints.

Limitations in available test methods pose an additional challenge in the coordination of definitions of resistance across sectors. For example, recommended phenotypic methods for the detection

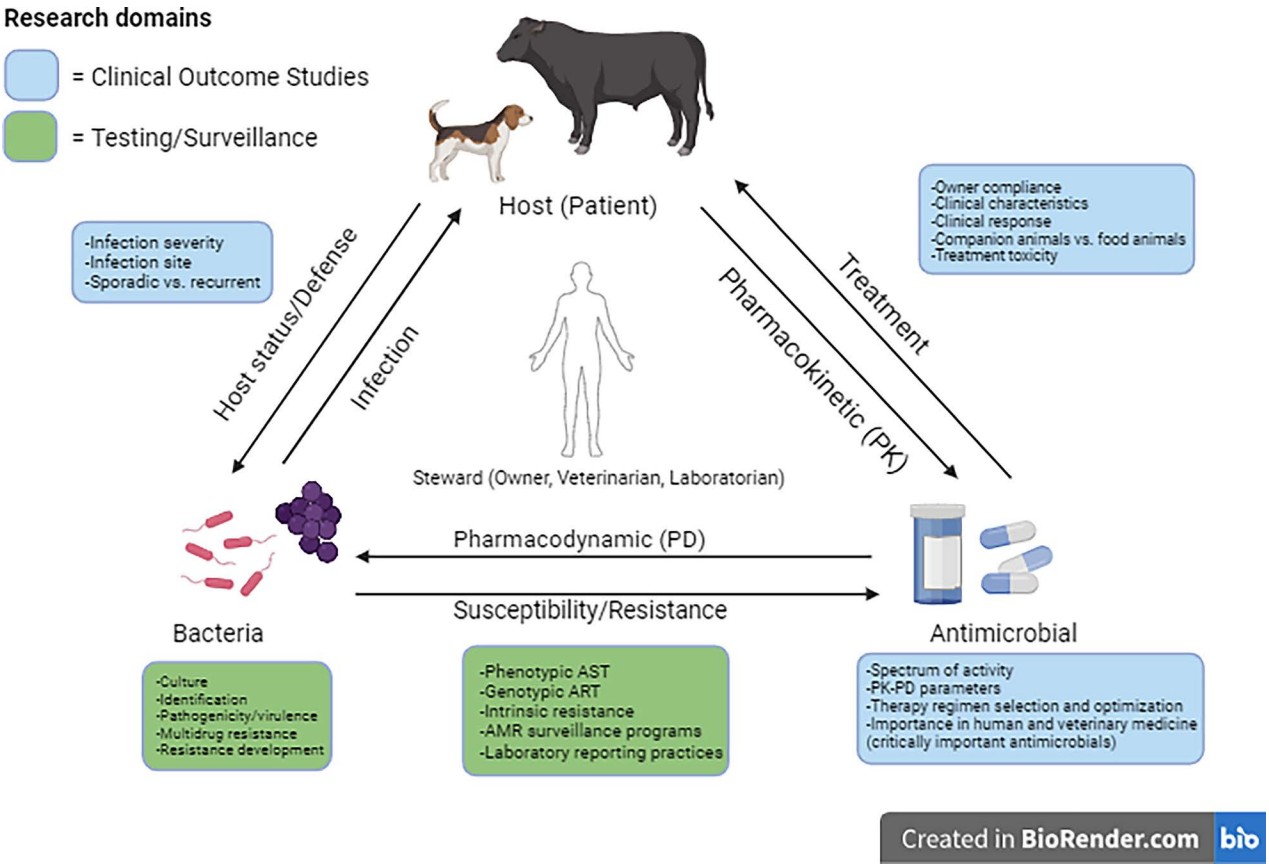


**Figure 1**—Research domains through the One Health antimicrobial resistance (AMR) lens. To minimize the adverse impact of AMR development, it is necessary to consider critically important antimicrobials and their use in human and veterinary medicine. ART = Antimicrobial resistance testing. AST = Antimicrobial susceptibility testing. Created with BioRender.com.

of CP-CRE, which are resistant to all β-lactams approved for use in veterinary medicine, are harmonized between Clinical and Laboratory Standards Institute (CLSI) human and veterinary testing criteria. However, animal species-specific breakpoints do not exist for carbapenems and their use in veterinary medicine is illegal in some jurisdictions. Therefore, testing, recognition of public health importance, and reporting practices vary across laboratories. Several commonly used commercial veterinary MIC methods test only imipenem, rather than meropenem, which is considered by CLSI, the CDC, and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) to represent the best balance of sensitivity and specificity when screening for the presence of carbapenamases.[12–14] While CP-CRE remains more commonly encountered in human medicine, reports among veterinary species are increasing globally, including documented nosocomial outbreaks within the US.[9,15] The lack of standardization for veterinary testing and/or reporting of carbapenems and lack of public health oversight for veterinary isolates are strong indicators that cases go unnoticed, furthering the potential for zoonotic transmission and global dissemination.

Due to the One Health significance of AMR and inconsistencies between sectors, collaboration, and adequate resources are necessary at the intersection of human, animal, and environmental health. Failure to collaborate and harmonize will undermine individual efforts in these areas. Public health reporting methods and standards should be harmonized to enable the appropriate interventions and ensure consistency of communication when it comes to AMR.[5,6]

## Veterinary Diagnostic Laboratories as Stewards

VDLs play a critical role in antimicrobial stewardship, although the power of laboratory communication to impact clinician behavior has not been fully explored in veterinary medicine. Often, VDLs report all antimicrobials tested on a susceptibility panel, regardless of their clinical appropriateness or the intrinsic resistance of an organism to a particular antimicrobial or antimicrobial class. Such practices should be discouraged, and veterinary clients should be aware that more results on an AST report are not indicative of better value or quality of test results. Several studies[16,17] in human medicine have demonstrated that laboratory reports directly impact physician treatment choices, termed Nudging in Microbiology Laboratory Evaluation (NIMBLE). Studies[17–19] of prescribers in human medicine have found that alterations to AST reports can improve the appropriateness of antimicrobial prescribing and that drugs listed on an AST report are more likely to be selected than those that are not included. A similar study of these reporting practices for both phenotypic AST and genotypic antimicrobial resistance testing (ART) in veterinary medicine may provide insight into ways that laboratories can help steer the reduction of and response to AMR in our animal populations.

## AST Standardization and Optimization

Phenotypic AST is the current gold standard for determining which antimicrobials are most appropriate for clinical use. Phenotypic AST is performed by broth microdilution or gradient diffusion, resulting in MIC values, or by disk diffusion, resulting in zone diameter (ZD) data. The MIC or ZD data provide information on the concentration of antimicrobials that have an inhibitory effect on bacterial growth, but these values alone are not indicative of clinical efficacy and must be interpreted using clinical breakpoints such as those developed by the CLSI or the EUCAST.[20,21] The clinical breakpoint allows the laboratory to provide the veterinarian with clinically predictive information on the likelihood of successful treatment of the patient's infection with a particular antimicrobial.

Genotypic test methods, such as whole genome sequencing (WGS), targeted sequencing, and metagenomic sequencing, are increasingly suggested as alternatives to traditional phenotypic methods.[22,23] Genotypic ART is the use of molecular methods (PCR, targeted sequencing, WGS, or metagenomic sequencing) to predict the presence of AMR genes.[22] In a clinical context, genotypic ART is used to determine which antimicrobials should not be used, whereas phenotypic AST guides the clinician on which antimicrobials could be used. Genotypic ART methods are increasingly used in human laboratory medicine in conjunction with phenotypic AST, and it would be best practice for veterinary medicine to adopt the same model if genotypic methods are used. Still, the incorporation of these methods in veterinary diagnostic medicine is unclear due to the high cost associated with routine use and lack of outcome studies.

Although widely used as part of the diagnostic testing algorithm, there are still many gaps in veterinary phenotypic AST. Commercially produced AST panels used in human medicine are stringently regulated by the FDA. In contrast, there is no regulation of veterinary AST panels, so the level of rigor and proven performance standards required for human AST panels is not required. While the potential harms of this are not well documented, the onus of verification and/or validation of test methodology is the sole responsibility of the VDL, which may have few resources for such activities. Recently, users of commercial AST panels were made aware of interlaboratory methodological discrepancies due to a lack of clarity in device instructions for use.[24] This highlights an additional need for more thorough evaluations of AST panels used in veterinary medicine to determine if resistance is being accurately detected. It also suggests more extensive training may be necessary for those performing veterinary AST, which might be accomplished through a variety of mechanisms, including proficiency testing, live training events, and webinars.

MIC-producing AST methods are often thought to be more desirable because of the numerical value and pharmacokinetic-pharmacodynamic (PK-PD)

relationship that can be assigned to the result. However, more data are not always better, and no single method will perform best for every species of bacteria. Disk diffusion testing continues to play an important role in veterinary AST and is frequently used in human laboratories. There is a need for additional research and data to expand veterinary disk diffusion antimicrobial options. Interpretive criteria are required to use ZD and MIC data to make predictions about clinical outcomes in patients.

A greater diversity of commercial AST methods is desirable to fit the needs of the veterinary diagnostic community. Currently, most VDLs perform AST using disk diffusion or broth microdilution.[7] Disk diffusion is highly standardized and has been widely used for AST for many decades; however, many veterinary-specific MIC breakpoints have been published in CLSI VET01S without corresponding disk diffusion breakpoints, making MIC methods the only option available for laboratories seeking to utilize veterinary-specific breakpoints. A mixture of available AST methodologies is necessary when testing diverse bacterial species, and careful consideration must be given to which reference methods are most appropriate for use.

Some fastidious bacteria (e.g., *Pasteurellaceae*) grow better on a solid medium, such as those used in disk diffusion, agar dilution, or gradient diffusion methodologies. Bacterial isolates that are growing well will produce more accurate, reproducible results. Manufacturer claims of utility for fastidious bacteria do not ensure the appropriateness of the method recommended for use.[25] Solid medium data for more fastidious bacterial species are just as powerful as broth microdilution methods, because the method is more appropriate and reproducible for that bacterium. Such test methods can then be easily and affordably implemented in a diagnostic setting, and AST findings generated from these studies can be used to help develop consensus data for standards development. In many cases, only a few antimicrobials are necessary for routine testing due to predictable susceptibility patterns of an organism in line with recommended first-line treatment recommendations. In such cases, an entire broth microdilution panel is not necessary and the flexibility to choose a small number of antimicrobials for a disk diffusion method will be more affordable. Limiting the number of antimicrobials tested and reported directly supports stewardship by focusing attention on the most appropriate narrow-spectrum antimicrobial.[16]

Phenotypic to genotypic correlation studies are powerful and potentially impactful for diagnostic medicine. Initial genotypic research studies should include both phenotypic and genotypic profiling. Agreement between testing will corroborate results, and disagreements will identify limitations of each test method or identify areas requiring additional research. Currently, there are 2 national veterinary programs geared at phenotypic to genotypic correlation studies.[26–28] Beyond phenotypic to genotypic correlation, these programs help laboratories compare methods and may help find areas of test method variability.[24] It should be noted that while important, surveillance data may be biased toward bacterial species with acquired resistance mechanisms (non-wild–type populations) due to isolation from clinically ill animals, which may skew resistance rates.

## AST in Research

Quality control (QC) should be woven into all tasks performed to ensure consistency, quality of test results, and competency of personnel. QC must be included in research studies with procedures intended to be used in a diagnostic setting to ensure that results are consistent, reproducible, and directly translatable to practice in the laboratory and clinical setting. QC is necessary regardless of whether a genotypic or phenotypic approach is used.[21,29] In the case of phenotypic AST, users must refer to the interpretive criteria of choice (CLSI and EUCAST) to determine which QC organisms are appropriate for a particular antimicrobial and organism.[21] Appropriate QC for genotypic methods will depend on the approach used. For PCR, positive and negative controls along with a dilution series and tests of sensitivity and specificity are required.[30] WGS and metagenomic approaches will have different requirements for QC.[29,31,32] No international standards have been developed for quality assurance, but many proposed guidelines are available and should be evaluated for use in these genotypic ART studies.[29,31,32]

Researchers must be aware that the methods used for phenotypic AST should only be used per manufacturer instructions for use and/or CLSI or EUCAST standards. Once altered, the methods are unstandardized and cannot be accurately interpreted by the interpretive criteria chosen (CLSI and EUCAST).[21] Whether contracting research to a diagnostic laboratory or performing the work in a traditional research setting, all parties should be aware of the exact methods used for testing and report any modifications to the manufacturer's instructions or other standardized methods.[21]

When mining retrospective data, it is important to review the precise methods used for AST performance, ideally comparing the laboratory protocol to the manufacturer's instructions for use and interpretive standards used. CLSI and EUCAST clinical breakpoints are updated periodically as more literature and clinical outcome data are generated. Some of the breakpoint changes are major and will dramatically affect the number of organisms that are classified as resistant going forward. For example, the 2013 introduction of feline-specific amoxicillin-clavulanate breakpoints for *Escherichia coli* isolated from skin and soft tissue or urine resulted in all *E coli* isolated from cats being categorized as resistant.[33] Previous data may have relied on extrapolation of human-specific breakpoints that more closely predict the presence of AMR genes in *E coli* but do not adequately predict the clinical success of using amoxicillin-clavulanate to treat systemic infections in cats. In 2020, based on new data and a reanalysis of existing data, UTI-specific feline breakpoints were revised to align with

the human-specific breakpoints and dog-specific urinary tract infection breakpoints.[34] A hypothetical retrospective study analyzing resistance trends over a 10-year period from 2012 to 2021 would, therefore, exhibit wild variation in the resistance rates. Depending on the time range of data collection, it might be necessary to harmonize the interpretation of the MIC or ZD results using a single edition of the interpretive criteria standard.[21] In addition, AST system manufacturers periodically update the dilution ranges on commercially available MIC plates, and older MIC results may not include test dilutions that correlate with current breakpoints. It is important to note that current and recently archived editions of CLSI VET01S and CLSI M100 are publicly available for free online at the CLSI website, so all researchers have open access to the most current standards.

## Clinical Breakpoints

Clinical breakpoints are expressed as susceptible, susceptible dose-dependent, intermediate, resistant, or nonsusceptible.[12,35] Animal species-specific clinical breakpoints have yet to be established for many antimicrobials while some existing breakpoints require re-examination using current methods for breakpoint determination. Modern breakpoint evaluation is primarily informed by 3 types of data: PK-PD data, microbiological AST data, and clinical outcomes data.[36,37] Detailed information about wild-type distributions, PK-PD modeling, and clinical cutoffs have been described in-detail elsewhere.[21,38–40]

Clinical cutoffs, determined by clinical outcome studies, play an important role in the establishm ent of CLSI breakpoints for humans but are infrequent in veterinary medicine. In the absence of outcome data, PK-PD data become increasingly important for setting veterinary speciesspecific breakpoints. However, PKPD data are sparse or incomplete for some antimicrobial drugs and in many species of animals, leaving laboratories to rely on extrapolation of breakpoints between animal species or humans. Additionally, based on a label or commonly used dose, PK-PD data may indicate a breakpoint that would split the wild-type population of bacteria, meaning that bacteria without an acquired resistance mechanism would be categorized as resistant to an antimicrobial due to the pharmacokinetic limitations in a particular animal species.[38] However, setting a breakpoint in the wild-type results in poor AST reproducibility, with isolates lacking acquired resistance mechanisms yielding variable results of susceptible or resistant if replicate testing were to be performed. Such reproducibility and accuracy issues have negative impacts on patients, AST method validation, and surveillance efforts. One solution to this problem may be through dose optimization that would allow for breakpoints that categorize all isolates within the wild-type population as Susceptible. Although frequently considered in human breakpoint evaluations and clinical practice, dose optimization is yet to be a widely accepted approach in veterinary medicine due to concerns about off-label use, especially in food animals. An additional limitation is the increased difficulty posed by administering drugs more frequently or parenterally. MIC and ZD distributions are also relied on but sometimes sufficient microbiological data have not been generated to determine the range of MICs demonstrated by the wild-type population of a bacterial species, making dose optimization and breakpoint determination a challenge.

When determining interpretive criteria for CLSI standards and guidelines, document developers rely on published studies or studies brought forth by sponsors.[41] It cannot be overemphasized that these studies must be conducted using standardized AST methods. For these studies to be most impactful and useful for CLSI or other standards setting organizations to consider for inclusion, consistent methods with appropriate QC, clear citations regarding which method was used for testing, atmospheric conditions, and appropriate test media must be used.[21,37,41] Importantly, as new CLSI and EUCAST clinical breakpoints are adopted or updated, it is imperative that interpretive criteria used in VDLs are updated; this is particularly critical when using automated AST instruments. It is imperative that result interpretations from automatic AST readers are also compared against a standard to ensure that correct interpretations were applied. Many users implicitly trust instrument software to provide accurate results; however, user review and validation should always be woven into software adoption and subsequent updates.[42] A recent survey[43] found that even in human laboratories the most updated CLSI breakpoints are not always used. New requirements from the College of American Pathologists will require updates to be made within 3 years of a new breakpoint development; however, these requirements are not in place for veterinary medicine.[44]

## Clinical Outcome Studies

Controlled clinical outcome studies regarding the efficacy of antimicrobial treatment protocols are scarce for veterinary medicine. Outcome studies would help define effective treatment regimens for different types of infections and thereby improve antimicrobial stewardship by providing evidence for treatment recommendations. In human medicine, large retrospective cohort studies[45–47] have been conducted to determine which CLSI breakpoints accurately predict response to selected antimicrobials, further validating developed breakpoints. In veterinary medicine, these studies would optimally occur in large teaching hospitals associated with a VDL, but VDLs could also partner with local clinics to establish, revise, or validate existing clinical breakpoints. Although more expensive to conduct, prospective studies using defined treatment regimens in a hospital setting would also be highly beneficial for the collection of phenotypic AST data.

Transparent AMU reporting should be encouraged and tools supporting collection and analysis

of these data should be developed.[48] AMU reporting supports studies targeting prescribing practices and is valuable for the development of antimicrobial stewardship programs because evidence of inappropriate selection may help improve treatment choice.[48–50] Several studies[49,51,52] have found inappropriate selection of a third-generation cephalosporin over peer-reviewed first-line treatment options; interventions after targeted training were successful at minimizing inappropriate treatment selection.

Beyond treatment outcomes, studies demonstrating the clinical utility of rapid genotypic ART methods in conjunction with phenotypic AST are necessary to prove genotypic ART provides a faster response, better patient outcomes, reduced treatment costs, and reduction of AMR in veterinary medicine.[53] Commercial genotypic ART platforms to detect MDROs are available and have been used with success in human medicine. Because MDROs have One Health importance, these assays, such as those to detect CP-CREs, could be adapted for use with appropriate testing and infection control response based on positive test results. These test methods could help to minimize the spread of nosocomial infections in veterinary hospital settings. It has been suggested that de-escalation of antimicrobial therapies is a sufficient justification for use of such test systems; however, other considerations may include reduction in length of hospital stays, reduced patient mortality, and improved clinical decision-making.[53]

## Education and Training

The education of veterinarians and veterinary students on appropriate AMU is a critical need in veterinary medicine. Targeted education has the potential to improve antimicrobial prescribing practices.[49,50,54] Studies focused on development of training programs and behavior modification outcomes are necessary to further improve current strategies and expand training capacity will help shape the future of veterinary training.

Beyond research, there is a critical need for training veterinary microbiology specialists and microbiologists working in the laboratory. Due to retirements, changing laboratory test needs, and the small number of training programs, VDLs are experiencing a shortage of trained laboratorians and specialty-trained veterinary microbiologists.[55] Funding for veterinary microbiology training programs is necessary to ensure continued workforce development and expertise is available to VDLs and veterinarians.

## Path Forward

Advances in test methodologies, phenotypic AST interpretive criteria, clinical outcomes, and surveillance efforts will ultimately improve patient care and preserve the efficacy of critically important antimicrobials.[3,4] Studies targeted at veterinary medicine must be prioritized for funding to ensure that antimicrobials remain effective for generations to come. Manufacturers of diagnostic tools must commit to partnership with VDLs to develop better test methods that capture the most current clinical breakpoints or recommended technologies while remaining affordable to clients. Education of veterinarians, veterinary students, and veterinary laboratory personnel is a critical need worthy of funding. Because these studies and training needs are necessarily interdisciplinary, effort should be made to include partnerships with diverse stakeholders across the One Health spectrum to ensure the most efficient and sustainable strategies are employed for use.

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