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Advances in infectious foci imaging using ^{99m}Tc radiolabelled antibiotics

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Abstract Conventional methods of infection diagnosis, relying on experimental tests and culture of organisms from infected foci have continued to developing new technologies and automation. Nuclear medicine is a reliable diagnostic technique capable to detect infectious foci in human disease. A wide range of radiolabeled agents have been evaluated for demonstrating their ability to distinguish microbial infectious lesions. New researches continue to be made on the use of radiolabeled antibiotics which as well as being highly specific in the diagnosis of infection would be useful in monitoring of disease treatment. Here, the new approaches of infection scintigraphic imaging by radiolabeled antibiotics are thoroughly discussed in order to assess and compare their diagnostic value as targeting imaging radiopharmaceuticals.

Keywords Infection · Radiopharmaceuticals · Infection specific imaging · Radiolabeled antibiotics · ^{99m}Tc · Foci

Introduction

Despite the advances in public health during the eighteenth and nineteenth centuries and the inauguration of immunization in the twentieth century, bacterial infection is among the most frequently causes of morbidity and mortality especially in developing countries [1]. The inflammatory reaction is a well-described sequence of events in response to an infection. In fact, inflammatory processes can be imagined in early phases, when anatomical changes are not yet apparent. But radiopharmaceuticals are able to detect the physiological and biochemical changes that occur during the early phases of inflammation. Infection can be considered as a special subcategory of inflammatory disease, i.e. an inflammatory reaction of the host in response to invasion by microorganisms [2]. Antibiotics are an indispensable part of modern medicine. The introduction of antibiotic-resistant mutants among bacteria is apparently inevitable, and results, within a few decades, in decreased efficacy and withdrawal of the antibiotic from widespread usage. The traditional answer to this problem has been to introduce new antibiotics that kill the resistant mutants. Unfortunately, after more than 50 years of success, the pharmaceutical industry is now producing too few antibiotics, particularly against Gram-negative organisms, to replace antibiotics that are no longer effective for many types of infection [3].

The ability to identify focal sites of infection in patients who do not present with localizing symptoms is a key step in delivering appropriate medical treatment. This is particularly critical in immune compromised patients, since signs and symptoms of infection may be minimized in patients with neutropenia [4]. There are so many different reasons that show why distinguishing between infection and inflammation becomes increasingly important. The population is ageing, the application of implants and transplants is increasing and the number of immune compromised patients is growing mainly because of frequent use of chemotherapeutic agents causing neutropenia. Additionally, the increased use of antibiotics leads to insensitivity for some of these pharmaceuticals [5].

Determining the “infection foci” in body sites are very important and lifesaving processes. Localization of deep seated infections such as osteomyelitis, endocarditis and intra-abdominal abscesses is still a challenging problem.

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Although another imaging techniques such as X-ray, computerized tomography (CT-scan), magnetic resonance imaging (MRI) and ultrasonography (US) might be helpful, but due to their limitations in determining anatomical changes especially in the early stages of the infection process, none of these techniques are specific for infection diagnosis. In addition, up to know, these techniques have not been capable of differentiating between inflammatory and infectious processes. In contrast, nuclear medicine technique can determine the exact location and the degree of disease in infectious processes based on physiologic and/or metabolic changes that are associated with these diseases rather than gross changes in the structure. The early detection of the infectious focus by radionuclide imaging helps both patient and physician to reduce the cost and the length of hospitalization [6].

The advantage of nuclear medicine is due to its ability in diagnosing particularly deep seated infections. It provides information on pathophysiological and patho-biochemical processes. In this respect it differs from other routine imaging procedures such as X-ray, CT and MRI, which supply information with high resolution on the morphological changes that occur in a specific disease. In addition, nuclear medicine technique allows whole-body imaging, whereas CT and MRI routinely focus on just a part of the body [7].

Nuclear medicine technique requires a reliable radiopharmaceutical that can selectively concentrate in sites of infection. Various ^{99m}Tc-labeled compounds have been developed for the scintigraphic detection of infection and sterile inflammation in humans. Unfortunately, these radiopharmaceuticals do not discriminate between infection and sterile inflammatory process, which is often of clinical importance. In recent years, the development of radiolabeled antibiotics for specific diagnosis of infection has received considerable attention, because of infection specificity of these radiopharmaceuticals [8]. Direct targeting of the locally present microorganisms is a new advance for improving the selectivity of radiopharmaceuticals for infection detection in nuclear medicine [9].

Single photon emission computed tomography (SPECT) shows function by means of a three dimensional activity distribution of a radioactive tracer, which was injected prior to the measurement. The principal values of SPECT are, as the result of the disposability of numerous single-photon radiopharmaceuticals, its broad clinical availability and its versatility for the everyday management of patients affected by several different conditions. Moreover, it is able to increase contrast and allow better delineation of pathologies than planar imaging. However, the main limitation of SPECT imaging is its poor anatomical information. Nearly 80 % of all radiopharmaceuticals used in clinical nuclear medicine are ^{99m}Tc compounds due to its extremely favorable physical and nuclear characteristics, its availability and low cost [10].

Antibiotic history

In 1945, Selman Waksman proposed that the word of antibiotic to the science environment for the first time [11]. Antibiotics are drugs of natural or synthetic origin that have the capability of killing or inhibiting of the growth of micro-organisms. Antibiotics are sufficiently non-toxic to the host so they are used as chemotherapeutic agents in the treatment of infectious diseases of humans, animals and plants [12]. Antibiotics are designed to support host defense in controlling infection. Most antibiotics used in human treatment were originated from natural materials produced by particular species of bacteria or fungi as a mechanism of competition to ensure their own survival [13].

Five basic against bacterial mechanisms of antibiotic action cells: inhibition of cell wall synthesis (most common mechanism), inhibition of protein synthesis (translation) (second largest class), alteration of cell membranes, inhibition of nucleic acid synthesis and antimetabolite activity. The major targets for the main classes of antibiotics include cell membranes, cell-wall biosynthesis enzymes and substrates, bacterial protein synthesis and bacterial nucleic acid replication and repair [14].

Antibiotics interfere with the growth of bacteria by three main ways: undermining the integrity of their cell wall, by interfering with bacterial protein synthesis and common metabolic pathways (Fig. 1). The terms bactericidal and bacteriostatic are broad categorizations, and may not apply for a given agent against all organisms, with certain antimicrobials being bactericidal for one bacterial pathogen but bacteriostatic for another. Bacteriostatic agents inhibit

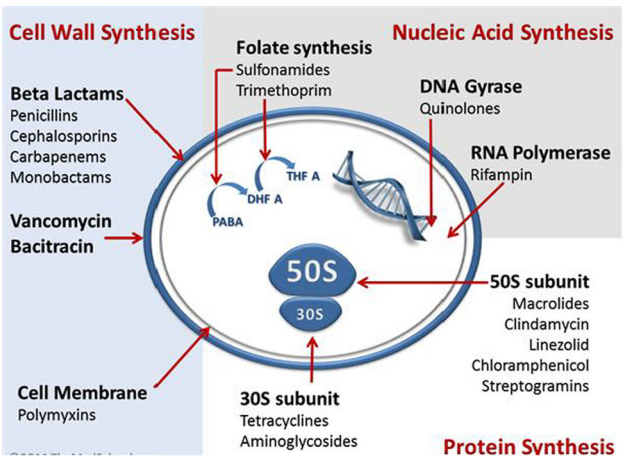


Fig. 1 Overview of antibiotics by mechanism (extracted from [17])

the growth of bacterial cells but do not kill them, whereas bactericidal agents kill the bacteria [15].

However, these categories are not absolute, since the killing effect of the drug varies with the test method and the species being tested [16]. Bactericidal antibiotics, such as the beta-lactams (including the cephalosporins, carbapenems, and cephems), glycopeptides (including vancomycin), fluoroquinolones, polymyxins, and the lipopeptide daptomycin, are often preferred for treatment of these diseases, particularly for cases of febrile neutropenia, meningitis, and endocarditis [13].

Radionuclide Technetium-99m (^{99m}Tc) is probably most widely used radionuclide due to its decay characteristics, low price and availability [18]. Another reason is because it is easy to coordinate with N, O and S which is convenient to label ^{99m}Tc with pharmaceuticals. The exactly chemical structures of these classical technetium pharmaceuticals, although some of them have been routinely used for more than 30 years, are still not known [19]. The use of radiolabeled antibiotics is fast emerging as a promising diagnostic test for the detection of infective foci, because of their specific binding to the bacterial component. The majority of other fluoroquinolone antibiotics, some of the cephalosporins and also other antibacterial agents were radiolabeled up to now for bacterial infection imaging with promising results [20]. It is believed that ^{99m}Tc involves the coordination to oxygen atom and nitrogen atoms of antibiotics to form negatively charged complexes. Possible binding of ceftriaxone with ^{99m}Tc [21] and binding structure of clinafloxacin with ^{99m}Tc $(CO)_3$ complex were proposed [22].

Quinolones based antibiotics

Quinolones are bactericidal agents that inhibit the replication and transcription of bacterial DNA, causing rapid cell death and are structurally related to nalidixic acid. Nalidixic acid is considered to be the predecessor of all members of the quinolone family, including the second, third and fourth generations commonly known as fluoroquinolones (Table 1; Fig. 2) [23, 24].

Fluoroquinolones are an important group of antibiotics which inhibit DNA gyrase enzyme and consequently inhibiting DNA synthesis. Fluoroquinolones categorized in four generations. Researchers divide the quinolones into generations based on their antibacterial spectrum [25]. The earlier-generation agents are, in general, more narrow-spectrum than the later ones, but no standard is employed to determine which drug belongs to which generation. The only universal standard applied is the grouping of the non-fluorinated drugs found within this class within the 'first-generation' heading. First-generation drugs achieve minimal serum levels. Second-generation quinolones have increased gram-negative and systemic activity. Third-generation drugs have expanded activity against gram-positive bacteria and atypical pathogens. Fourth-generation quinolone drugs add

Table 1 Characters of radiolabelled fluoroquinolones

Name	Generation	Radiochemical purity (%)	Stability	T/TN ratio	Type of animal	Half life (h)	Reference
Sparfloxacin	3rd	>95	>92 %	5.9	Rabbit	16–30	[34], [35], [25]
Enrofloxacin	2nd	>95	Moderately	4.2	Rat	4–6	[36], [37]
Pefloxacin	2nd	>98	>96 %	5.6	Mice	8.6	[38]
Lomefloxacin	2nd	>93	>80 %	6.5	Rat	7.75	[39]
Ofloxacin	2nd	>96	>80 %	4.3	Rat	4–5	[39]
Difloxacin	3rd	>98	>86 %	5.5	Rat	7.92	[38]
Moxifloxacin	4rd	>95	>84 %	6.8	Rabbit	12	[40]
Norfloxacin	2nd	>95	>92 %	6.9	Rat	3–4	[41]
Gemifloxacin	4rd	>97	>90.5 %	5	Rat	7	[42]
Rufloxacin	2nd	>98	>90 %	4	Rat	38	[43]
Clinafloxacin	4rd	>97	>81 %	5	Rat	5.5	[44]
Garenofloxacin	–	>97	>82 %	5	Rat	8	[45]
Gatifloxacin	4rd	>90	>87 %	4.5	Rat	7–14	[46]
Fleroxacin	4rd	>98	>90 %	5	Rat	10	[47]
Trovafloxacin	–	>97	>96 %	5	Rat	9.4–12.7	[48]
Sitafloxacin	–	>98	>81 %	18.5	Rat	7	[49]
Levofloxacin	3rd	>95	>95 %	3.96	Rat	6–8	[50]
Temafloxacin	–	>98	>90 %	5	Rat	11	[51]
BDOQCA	–	>97	>91 %	7.6	Mice	14	[52]

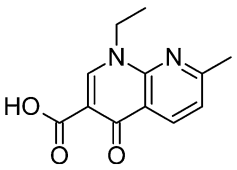


Fig. 2 Nalidixic acid structure

significant activity against anaerobes. The quinolones can be differentiated within classes based on their pharmacokinetic properties. The new classification can help family physicians prescribe these drugs appropriately.

^{99m}Tc-ciprofloxacin (^{99m}Tc-infecton)

Ciprofloxacin hydrochloride is a synthetic broad spectrum quinolone antibiotic which is absorbed by Gram-positive and Gram-negative bacteria and inhibits DNA synthesis by binding to bacterial DNA gyrase [26]. Ciprofloxacin binds reversibly to mammalian topoisomerase II but with 1000 fold lesser affinity [27]. Quinolones divided into four generations (Table 2) and inhibit two antibacterial key-enzymes, DNA-gyrase (topoisomerase II) and DNA topoisomerase IV. Ciprofloxacin is metabolized in the liver and eliminated by renal excretion.

The first clinical application of ^{99m}Tc-ciprofloxacin was reported by Vinjamuri et al. and the ability of ^{99m}Tc infecton imaging in comparison with radiolabeled white blood cell imaging for evaluating of bacterial infection, were investigated [28].

The authors demonstrated 84 % sensitivity and 96 % specificity of ^{99m}Tc-ciprofloxacin in contrast to 81 % sensitivity and 77 % specificity of white blood cell imaging. Following injection, only 20–30 % of ciprofloxacin is bound to plasma proteins and the agent becomes widely distributed throughout the body.

Ciprofloxacin has several advantages over radiolabeled leucocytes, and other methods for imaging infection, which include the following:

(a) Specificity for infection.
(b) Lack of bone marrow uptake, which is a significant advantage in imaging bone and joint and orthopedic prostheses infections.
(c) Ease and cost of preparation of the agent.
(d) Ex vivo labeling, which avoids contact with blood and hence the risk of acquiring blood borne infections such as HIV and hepatitis B and C.
(e) Independence of the host inflammatory response and neutrophil count and hence it can be used to image infections in immune compromised patients, including those who are neutron paenic, where culture is often negative and white blood cell imaging unreliable.
(f) Availability in a kit format with long shelf-life, making it user friendly and more widely available [29].

However, the low binding affinity of ^{99m}Tc-ciprofloxacin to bacteria and the risk of emerging antibiotic-resistant microorganisms make this radiopharmaceutical unattractive for imaging bacterial infections [30].

^{99m}Tc-infecton been extensively evaluated by many groups around the world in a wide range of scenarios. The availability of infecton in a kit form for local reconstitution and labeling, enabled a large scale multi center evaluation to be performed across 8 countries [26, 31]. In that study, which included a different range of infectious disease including endocarditis, tuberculosis, osteomyelitis and prosthetic joint infection, the radiopharmaceutical showed an overall sensitivity of 85.4 % and specificity of 81.7 % for the diagnosis of infection when classified by CDC, Duke or WHO criteria. The patients in this study were subjected to rigorous microbiological evaluation and in patients in whom infection could be confirmed by culture, as well as clinical criteria specificities of over 90 % were obtained. The radiopharmaceutical seemed to be particularly applicable in bone and joint infections including infected orthopaedic prosthesis and follow up studies have been performed since. The method of preparation and quality

Table 2 Characteristics of radiolabeled cephalosporins

Name	Generation	Radiochemical purity (%)	Stability	Half-life (h)	T/TN ratio	Type of animal	Reference
Ceftizoxime	3rd	>94	83 %	1–9	2.73	Rat	[55]
Cefuroxime	2nd	>98	>92 %	3.5	1.8	NR	[56], [57]
Cefotaxime	3rd	>92	>90 %	3.4	2.89	Mice	[58]
Ceftriaxone	3rd	>95	>90 %	25	5.6	Mice	[59], [60]
Ceftazidime	3rd	>95	>85 %	1.6–2	1.4	Mice	[61]
Cefoperazone	3rd	>97	NR	1.6–2.4	4.5	Rat	[62]
Cefepime	4th	>98	>98 %	2	8.4	Rat	[46]

NR not reported

control of many of the in house preparations of ^{99m}Tc-ciprofloxacin has led to controversy over the reliability of some of the published data [26].

Osteomyelitis in sickle cell disease is difficult to distinguish from bone infarction following sickle cell crisis. Bererhi [32], compared the use of infecton with three phase bone scanning using ^{99m}Tc MDP in 35 patients with sickle cell disease and suspected osteomyelitis by microbiological and clinical criteria. The sensitivity and specificity of infecton were 100 and 92 % respectively, compared to 88 and 64 % for bone scanning. Author of this review prepared the kit in house and evaluated the stability, Biodistribution and localization in the infectious foci and showed that the target/non-target ratio of the radiopharmaceutical is about 3.2 [33].

The structural features of the Tc=O complexes can be explained on the basis of the reported structural of Tc≡N. Technetium can have a number of oxidation states but the +V state is the most common in Tc≡N and Tc=O complexes with d^2 configuration. Infrared spectra of fluoroquinolones which have many common groups shows peaks on KBr in 3460 cm^{-1} due to OH of COOH group of carboxyl group absorbed in the region of 1685 cm^{-1}. The disappearance of peak due to OH in spectra of ^{99m}Tc fluoroquinolones indicated the binding of technetium with hydroxyl oxygen to a lower frequency group. The shift of Tc=O frequency to a lower frequency of 1650 cm^{-1} indicated the binding of Technetium with carbonyl oxygen. The proposed structure of the ^{99m}Tc fluoroquinolone for example ^{99m}Tc-Sparfloxacin complex is shown in Fig. 3; [36]. The speculated structure of ^{99m}Tc-Fluoroquinolones with bidental ligand will have a square pyramidal geometry with ^{99m}Tc-Fluoroquinolone ratio of 1:2.

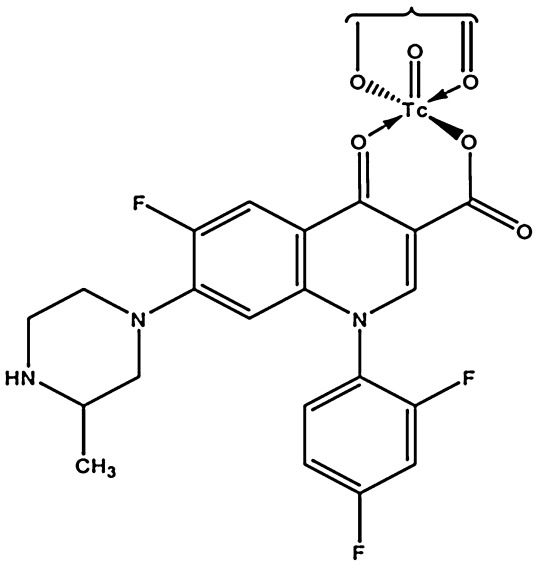


Fig. 3 Proposed radiolabeling site of fluoroquinolones [51]

For almost antibiotics, to optimize the labeling conditions, experiments were carried out by dissolving different amount of the antibiotics in distilled water, followed by the addition of varying amounts of reducing agent and in some cases different amounts of coligand and adjusting the pH. Then pertechnetate was added to the mixture and incubated in room temperature for a period of 10–30 min.

Cephalosporins

Recent developments in the chemistry and biology of *b*-lactam antibiotics which culminated with the introduction of several clinically useful classical and non-classical *b*-lactams have been most thrilling and highly rewarding. Cephalosporins are indicated for the prophylaxis and treatment of infections caused by bacteria susceptible to this particular form of antibiotic. First-generation cephalosporins are active predominantly against gram-positive bacteria, and successive generations have increased activity against gram-negative bacteria. Cephalosporins are bactericidal and have the same mode of action as other beta-lactam antibiotics (such as penicillins) but are less susceptible to penicillinases. Cephalosporins disrupt the synthesis of the peptidoglycan layer of bacterial cell walls. The peptidoglycan layer is important for cell wall structural integrity. The final transpeptidation step in the synthesis of the peptidoglycan is facilitated by trans peptidases known as penicillin-binding proteins (PBPs). The cephalosporin nucleus can be modified to gain different properties. Cephalosporins are sometimes grouped into "generations" by their antimicrobial properties. The first cephalosporins were designated first-generation cephalosporins, whereas, later, more extended-spectrum cephalosporins were classified as second-generation cephalosporins. Each newer generation has significantly greater gram-negative antimicrobial properties than the preceding generation, in most cases with decreased activity against gram-positive organisms. Fourth-generation cephalosporins, however, have true broad-spectrum activity [54].

It's worthy to say that the various complexes of ^{99m}Tc may be formed by interaction between electron donor atoms and reduced technetium. In order to form bonds with technetium, the structure must contain electron donors such as oxygen, nitrogen and sulfur. Although the exact structure of cephalosporins complex with ^{99m}Tc is not known, results showed that the labeled complex may be formed electron pairs of these atoms with reduced technetium that is +1 or +3 in the reduced states similar to other studies.

Radiolabeled anti tubercular agents

Tuberculosis is diagnosed by finding *Mycobacterium tuberculosis* bacteria in a clinical specimen taken from the

patient and the disease diagnosis depends on the clinical history, physical examination, a chest X-ray and on the results of radiological, immunological (ELISA) and microbiological tests or histo-pathological examinations of biopsy samples. It may also include a tuberculin skin test, other scans and X-rays, surgical biopsy. All these techniques have proven their nuclear utility but they suffer from one or other drawbacks.

Tuberculosis continues to be a devastating disease worldwide and is believed to be present in about one-third of the world's population. *Mycobacterial* infections have been shown to be increasing in number worldwide, mainly due to a global increase in developing countries, the increased number of patients with HIV infection and AIDS disease worldwide, an increasing the number of elderly patients and the emergence of multidrug resistant tuberculosis [63].

Although the etiological agent as well as tuberculosis pathogenesis is well known, the molecular mechanisms underlying the host defense to the bacilli remain elusive [64].

^{99m}*Tc–ethambutol*

A suitable ligand, ethambutol (EMB) with half-life of 3–4 h, that is, first line anti tubercular drug was chosen for detection as well as localization of the lesion using nuclear medicine modality. Causse et al. radiolabeled EMB with ^{99m}Tc for the first time in 1990 and demonstrated that the radiolabelling yield was 90 %. The low toxicity of ethambutol is well known. However they reported that ethambutol could be used as a radiopharmaceutical in the study of renal function [65]. In 2005 Verma et al. reported that ^{99m}Tc–EMB can be used in humans for tubercular imaging. Therefore it was concluded that this radiolabeled agent can be used for detection and follow up of tuberculosis lesions in patients especially to determine the treatment endpoint of anti-tuberculosis drugs [66].

^{99m}*Tc–isoniazid*

Isoniazid is another anti-tuberculosis agent with half-life of 1–2 h, that binds to mycolic acid in the cell walls of living *Mycobacteria* [26]. Isoniazid was successfully radiolabelled with ^{99m}Tc with the radiochemical purity of 95 %. It is shown to be stable, reproducible and safe preparation having specific accumulation in *Mycobacterium*. [67].

^{99m}*Tc–rifampicin*

Rifampicin (RMP) with a half-life of 1.5–5 h is a new antibiotic of rifampicin group intended for the management of tuberculosis. It was labeled with ^{99m}Tc with the radiochemical purity of 98 %. Authors reported that initially in the infected muscle of the artificially infected rats the activity was lower but after 90 min it went up to 18.3 from 5.95 % and the T/NT ratio is 7.3, 90 min post injection which was 2.38 initially. [68].

Other radiolabeled antibiotics

^{99m}*Tc–vancomycin*

Vancomycin with half-life of 4–6 h, is active against *Staphylococci*, *Streptococcus*, etc. [69]. The antibiotic was labeled with ^{99m}Tc and its biological activity was investigated in a model of intramuscular inflammation or infection in rats by Roohi et al. They reported higher uptake of ^{99m}Tc–vancomycin in *S. aureus* infected animals than that in turpentine-inflamed rats. It was found T/NT was 5 at 1 h post injection. As for sterile infected muscle the T/NT ratio was 1.5 at 1 h. [70].

^{99m}*Tc–kanamycin*

Kanamycin sulfate with half-life of 2.5 h was labeled with ^{99m}Tc by Roohi et al. In their study, ^{99m}Tc–kanamycin was administrated in infected rats with *S. aureus* ATCC 25923. In vivo experimental results demonstrated that the highest obtained T/NT ratio of ^{99m}Tc–kanamycin was 2.5 [71].

Patients with neoplastic diseases are at significant risk for such infections as a result of their underlying illness and its therapy [72, 73].

^{99m}*Tc–fluconazole*

Fluconazole with half-life of 30 h was successfully labeled with ^{99m}Tc by Lupetti et al. This labeled compound successfully detected infections with *Candida albicans* but not bacterial infections or sterile inflammatory sites in animals [74].^{99m}Tc–fluconazole detected *C. albicans* infections with T/NT = 3.6 without visualizing bacterial infections (T/NT = 1.3) or sterile inflammatory processes (heat-killed *C. albicans T/NT = 1.3* (Table 3).

Conclusion and future perspectives

Development of Infection imaging agent will help physicians in monitoring the success of infection antimicrobial therapy with multi drug resistant pathogens. New technology of nuclear medicine offers an attractive tool for diagnosis of focal infections due to its sensitivity based on pathophysiological and patho-biochemical processes [75]. ^{99m}Tc-labeled antibiotics make them the infection seeking agent of choice. Some of them have now been successfully

Table 3 Antibiotics studied for infection imaging

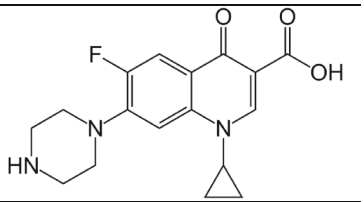
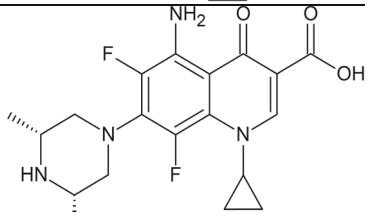
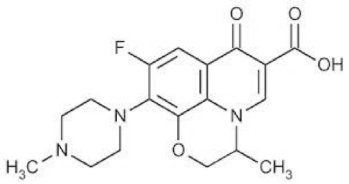
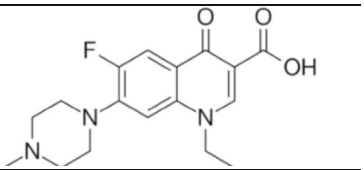
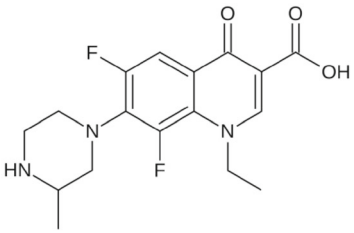
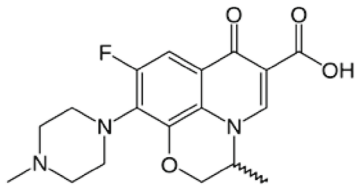
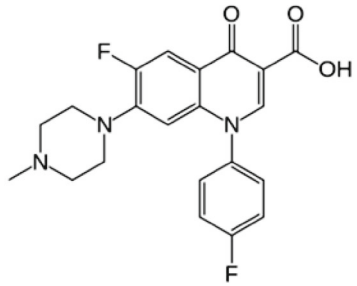
Antibiotic	Anti-microbial Group	Microorganism Affected	Localization	Reference	Structure
Ciprofloxacin	Fluoro-quinolone second generation	S. aureus	Binding to bacterial DNA gyrase	Vinjamuri 1996 [28]	
Sparfloxacin	Fluoro-quinolon third generation	S. aureus	Inhibition of bacterial DNA gyrase	Singh 2003 [34], Motaleb 2009 [35]	
Enroflaxacin	Fluoro-quinolone Second generation	S. aureus, Candida albicans	Inhibition of bacterial DNA gyrase	Siaens 2004 [36]	
Pefloxacin	Fluoro-quinolone Second generation	Escherichia coli	Inhibition of bacterial DNA gyrase	El-Ghany 2005 [1]	
Lomefloxacin	Fluoro-quinolone Second generation	S. aureus	Inhibition of bacterial DNA gyrase	Motaleb 2007 [39]	
Ofloxacin	Fluoro-quinolone Second generation	S. aureus	Inhibition of bacterial DNA gyrase	Motaleb 2007 [62]	
Difloxacin	Fluoro-quinolone Third generation	S. aureus	Inhibition of bacterial DNA gyrase	Motaleb 2010 [38]	

Table 3 continued

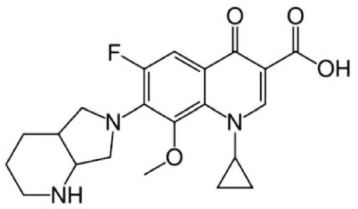
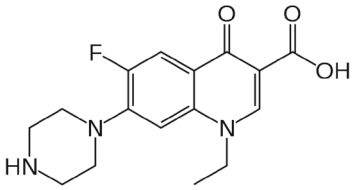
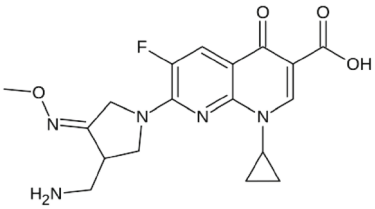
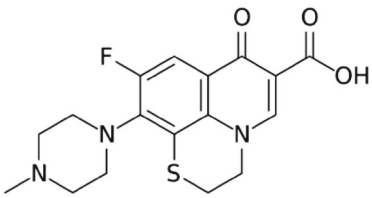
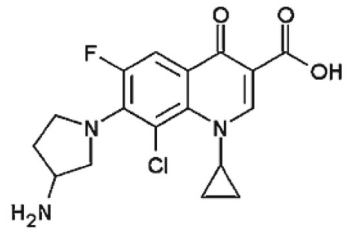
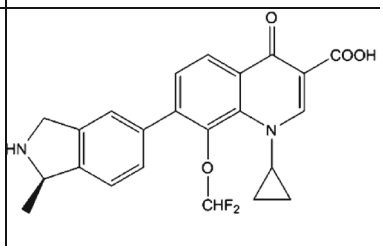
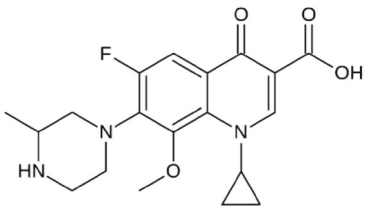
Antibiotic	Anti-microbial Group	Microorganism Affected	Localization	Reference	Structure
Moxifloxacin	Fluoro-quinolone Fourth generation	Escherichia coli	Inhibition of bacterial DNA gyrase	Chattopadhyay 2010 [40]	
Norfloxacin	Fluoro-quinolone Second generation	S. aureus	Inhibition of bacterial DNA gyrase	Ibrahim 2010 [41]	
Gemifloxacin	Fluoro-quinolone Fourth generation	S. pneumoniae	Inhibition of bacterial DNA gyrase	Shah 2011 [42]	
Rufloxacin	Fluoro-quinolone Second generation	S. aureus	Inhibition of bacterial DNA gyrase	Shah 2011 [43]	
Clinafloxacin	Fluoro-quinolone Fourth generation	S. aureus	Inhibition of bacterial DNA gyrase	Shah 2011 [44]	
Garenofloxacin	Fluoro-quinolone	multi-resistant S. aureus (MRSA) penicillin-resistant Streptococci (PRSC)	Inhibition of bacterial DNA gyrase	Shah 2011 [45]	
Gatifloxacin	Fluoro-quinolone Fourth generation	Escherichia coli	Inhibition of bacterial DNA gyrase	Motaleb 2011 [46]	

Table 3 continued

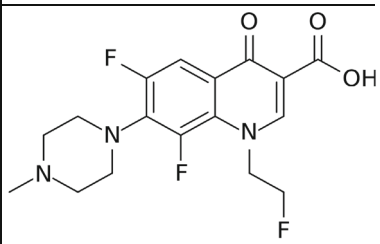
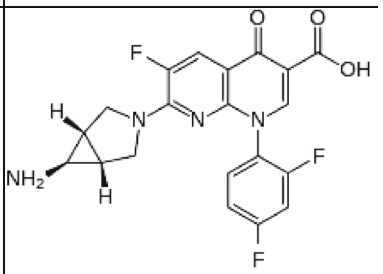
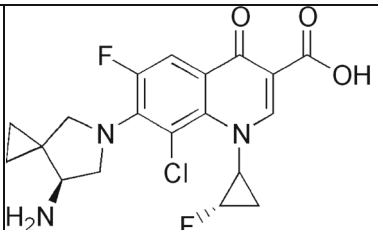
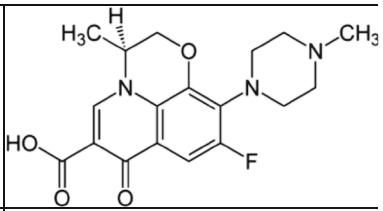
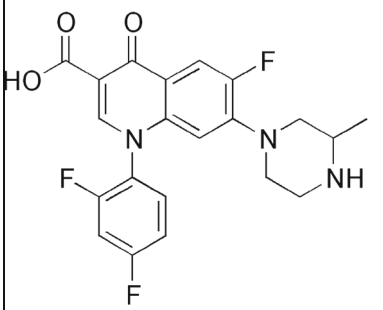
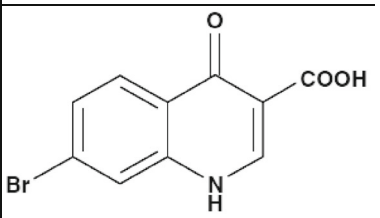
Antibiotic	Anti-microbial Group	Microorganism Affected	Localization	Reference	Structure
Fleroxacin	Fluoro-quinolone	Escherichia coli	Inhibition of bacterial DNA gyrase	Shah 2011 [47]	
Trovafloxacin	Fluoro-quinolone	multi-resistant S. aureus (MRSA)	Inhibition of bacterial DNA gyrase	Shah 2011 [48]	
Sitafloxacin	Fluoro-quinolone	S. aureus	Inhibition of bacterial DNA gyrase	Qaiser 2010 [49]	
Levofloxacin	Fluoro-quinolone Third generation	S. aureus	Inhibition of bacterial DNA gyrase	Naqvi 2012 [50]	
Temafloxacin	Fluoro-quinolone	S.aureus, Moraxella catarrhalis, Haemophilus influenzae, Legionella pneumophila, Klebsiella pneumoniae, Streptococci pneumoniae, Streptococcus pyogenes	Inhibition of bacterial DNA gyrase	Shah 2013 [51]	
BDOQCA	quinolone	Escherichia coli	Inhibition of bacterial DNA gyrase	Al-wabli 2011 [52]	

Table 3 continued

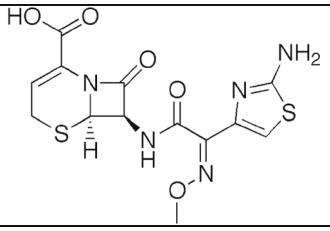
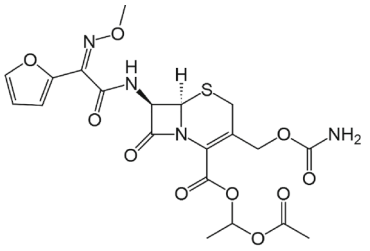
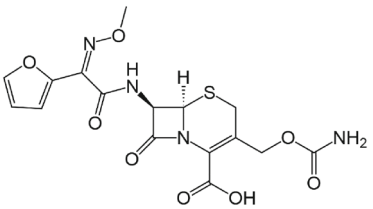
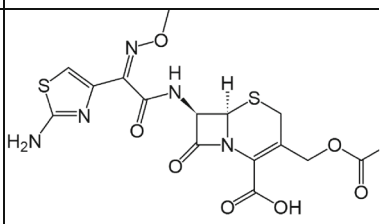
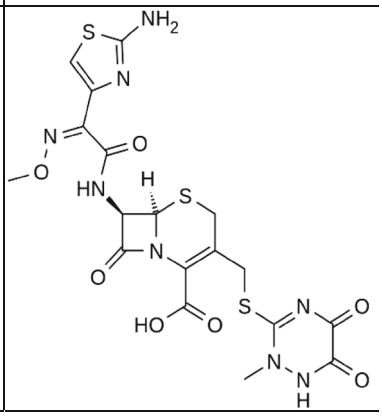
Antibiotic	Anti-microbial Group	Microorganism Affected	Localization	Reference	Structure
Ceftizoxime	Cephalosporin Third generation	S. aureus, Escherichia coli	Inhibition of the trans-peptidase enzyme	Barreto 2005 [53], Diniz 2005 [55]	
Cefuroxime	Cephalosporin Second generation	Escherichia coli	Inhibition of the trans-peptidase enzyme	Lambrecht 2008 [56], Chattopadhyay 2012 [57]	Cefuroxime Axetile:  Cefuroxime: 
Cefotaxime	Cephalosporin Third generation	S. aureus	Inhibition of the trans-peptidase enzyme	Mirshojaei 2011 [58]	
Ceftriaxone	Cephalosporin Third generation	Escherichia coli	Inhibition of the trans-peptidase enzyme	Mostafa 2010 [59], Fazli 2012 [60]	

Table 3 continued

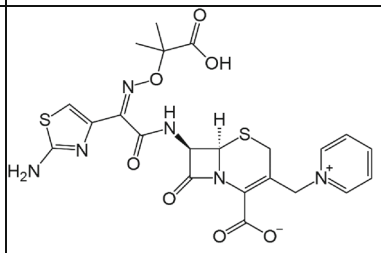
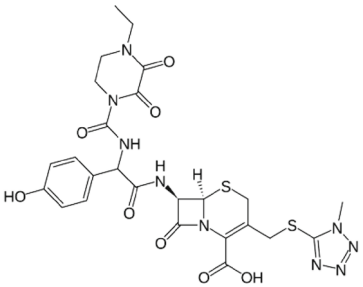
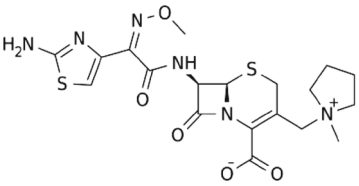
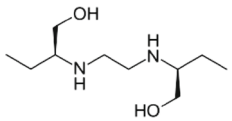
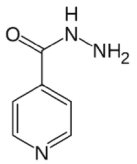
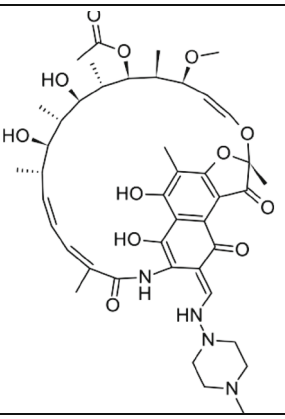
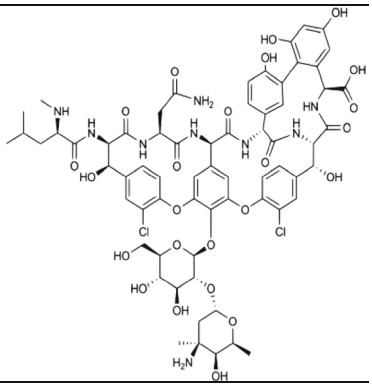
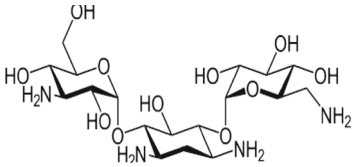
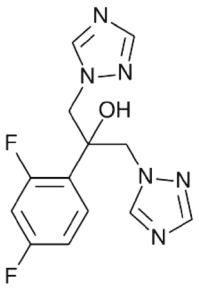
Antibiotic	Anti-microbial Group	Microorganism Affected	Localization	Reference	Structure
Ceftazidime	Cephalosporin Third generation	S. aureus	Inhibition of the trans-peptidase enzyme	Mirshojaei 2013 [61]	
Cefoperazone	Cephalosporin Third generation	S. aureus	Inhibition of the trans-peptidase enzyme	Motaleb 2007 [62]	
Cefepime	Cephalosporin Fourth generation	Escherichia coli	Inhibition of the trans-peptidase enzyme	Motaleb 2011 [46]	
Ethambutol	Antituberclosis	mycobacteria	Inhibition of the trans-peptidase enzyme	Causse 1990 [65], Verma 2005 [66]	
Isonizid	Antituberclosis	mycobacteria	inhibits the P450 system	Singh 2003 [67]	
Rifampicin	Antituberclosis	multi-resistant S. aureus (MRSA), listeria, neisseria, gonorrhoeae, haemophilus, influenza, legionella pneumophila	Inhibiting the transcription and succeeding translation to proteins	Shah 2010 [68]	

Table 3 continued

Antibiotic	Anti-microbial Group	Microorganism Affected	Localization	Reference	Structure
Vancomycin	Vancomycin	multi-resistant S. aureus MRSA, *S. aureus, S. epidermidis*	bactericidal: disrupts peptioglycan cross-linkage	Roohi 2005 [70]	
Kanamycin	Aminoglycoside	*E. coli*, *Proteus spp., Serratia marcescens*, *Klebsiella pneumoniae*	inhibiting translocation of protein synthesis	Roohi 2006 [71]	
Fluconazole	Antifungal	Candida albicans	Inhibiting the synthesis of ergosterol	Lupetti 2002 [74]	

applied in clinical settings and further evaluation with different types on infection in human will sort out the future for the promising compound. Probes for this application are reliable radiopharmaceuticals. It is not only enough for the radiopharmaceutical to be fast accumulating and sensitive in infection imaging; it should also show high specificity that can localize in site of infection. Infections and sterile inflammation discriminating by imaging with radiopharmaceuticals and antimicrobial therapy monitoring based on bacteria number is really unique considering the fact that neither CT nor MRI is able to detect micro-organisms. In clinical setting, it is important to correlate functional scintigraphic studies with anatomical imaging which is another progress in infection imaging by improving the instrumentations. Recently, single-photon emission computed tomography (SPECT) as well as Positron emission tomography (PET) provides images for direct correlation to anatomical modalities such as CT and MRI. These fusion methods include side by side, software fusion. It is believed that fusion imaging would increase the specificity of the physiologic modality and increase the sensitivity of anatomical modalities [76].

In this manuscript, recent improvement in developing ^{99m}Tc-labeled antibiotics was completely reviewed. All of these radiopharmaceuticals are designed for direct intravenous injection and aimed to target infectious and inflammatory cells or invading pathogens but each of them with their own advantages and disadvantageous. Radiolabeling different kinds of antibiotics will help to identify focal sites of infection in patients, help to develop more efficient antibiotics and minimize the side effects and toxicity of antibiotics by choosing right and more potent antibiotics for patient and finally reduce the cost of treatment. As mentioned here, various conventional radiopharmaceuticals which are basically on the uptake mechanism of targeting host inflammatory response are not specific for infection imaging. In contrast, the use of radiopharmaceuticals for specific targeting of microorganisms responsible for infection, have been proposed. In this respect, radiolabeled antibiotics by specific binding to the bacterial portions have the potential to distinguish infection at the early stage of diseases from noninfectious inflammation. Author suggest that, future progress related to ^{99m}Tc-labeled antibiotics will be pursued for imaging of

different kinds of infection and antibiotics with wide promising properties as the infection imaging agents have the ability to be used in clinical usages in patients with suspected infections for more accurate diagnosis.

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