Research Article

An *in vitro* antimicrobial evaluation of Cobalt (II) complexes of Amoxicillin, Ciprofloxacin, Norfloxacin and Ketoconazole

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Abstract

Objective: The objective of the present study was to evaluate *in vitro* antimicrobial properties of cobalt (II) complexes of amoxicillin, ciprofloxacin, norfloxacin and ketoconazole. **Materials and methods**: The ultra violet - visible absorption spectra and fourier transform infrared specta of the ligand and the complexes were obtained and helped to elucidate the bonding and antimicrobial properties. The ligands and their complexes were subjected to antimicrobial screening against common bacterial strains *E. coli*, *Staphylococcus aureus*, *Salmonella typhae*, *candida* and *Vibro*. **Results**: The results showed that the complexes are more effective as antibacterial agents as compared to the uncomplexed ligand (control) in all the range of concentrations used. The FTIR data indicated complexation of cobalt(II) ion to the ligands as shown by the shift of vibration spectra at C=N and C=O and appearance of new bands indicating M-O and M-N bands. **Conclusion**: Based on the outcome of this study, it is recommended that cobalt(II) complexes of ciprofloxacin, amoxicillin, ketoconazole and norfloxacin should be used in the treatment of infections caused by these micro-organisms.

Keywords: Cobalt(II) ions, micro-organisms, minimum inhibitory concentration, antimicrobial agent

Introduction

The use of drugs for the prevention, control and eradication of diseases is an age long practice and various substances have since been systematically applied in various disease management, control and eradication. Studies have shown that drug combination which offers essentially a good number of novel properties like increased activity, synergistic activity, decreased drug resistance, increased spectrum activity and decreased cost, number of needed doses and toxicity (Yu et al., 2003; Jimenez et al., 2005).

The resistance of common antibiotic is a fast growing phenomenon in medicine and has presented itself as a serious concern for drug users and administrators. The first line antibiotics have been worst hit and this therefore necessitates the search for drugs that the mode of action is different from the drug with established resistance. Studies have shown that a good number of metal complexes accelerate drug bioactivity a consequence of the ease of cleavage of the bond between the ligand and the metal cation (Unemo, 2012; Kostova and Momekov, 2006; Juan and Caredmy, 2001). The bioactivity of a chemotherapeutic agent upon co-ordination with a metal ion is improved and is highly dependent on the donor ability, sequence and nature of the metal ion. These calls for the synthesis of new antimicrobial agent for the treatment of resistant bacteria agents and metal complexes of such drugs are preferred from various new findings on the biochemical and chemical action of metal complexes in chemotherapy (Starnino et al., 2012; Chen et al., 2001).

Recent works (Singh et al., 2012) on metal complexes have proved that co-ordination of a drug to metal ion enhances its activity and in many cases the metal complex possesses such activity that the parent antibiotic does not have, we are motivated to study the antimicrobial properties of cobalt (II) complexes of ciprofloxacin, norfloxacin amoxicillin and ketoconazole. The chemical structures of the studied compounds are shown in Figures 1-4.

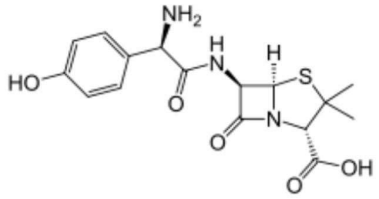

Figure 4. Structure of norfloxacin

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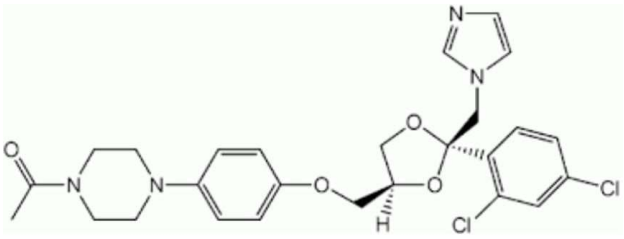

Figure 2. Structure of ketoconazole

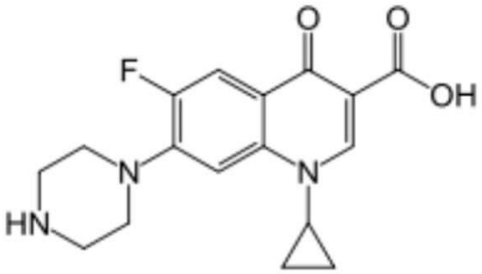

Figure 3. Structure of ciprofloxacin

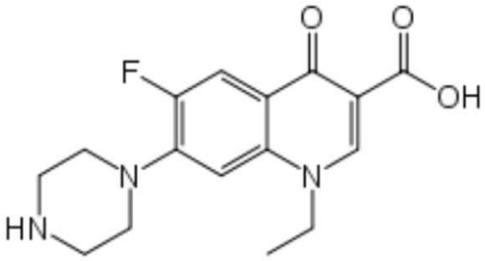

Figure 4. Structure of norfloxacin

Materials and methods

Reagents

Cobalt (II) chloride hexahydrate ($CoCl_2.6H_2O$) from Merck, solutions for antibacterial studies and sensitivity tests: ketoconazole, ciprofloxacin, amoxicillin and ampiclox (hovid); mueller-Hinton agar (TM-media laboratory PVT ltd, India), nutrient agar (TM-media laboratory PVT ltd, India), nutrient broth (TM-media laboratory PVT ltd, India), analytical weighing balance (Adam equipment co Ltd, UK). The chemicals used including absolute Ethanol, 99 % (BDH chemical Ltd Poole, England) and acetone, 70 % (JHD chemical laboratory Ltd) were of analytical grade and never purified further.

Instruments

Autoclave (Alpha Laboratory, Ltd) model NL280-A; oven (new life medical instrument England) model: nl-9052-1; pressure cooker (Crown Star Atsago, India Instruments Pvt. Ltd, India), water bath (helmreasinn multi-purpose) model:DK420; ultraviolet(UV) spectrophotometer (Genesis 10S UV-Vis spectrophotometer); infrared Perkin-Elmer FTIR-8400S Fourier transform infrared spectrophotometer (Shimadzu, Japan) in the range of 4000-400cm^{-1} as KBr disks.

Experimental

Sample collection and confirmation

The relevance of these organisms *staphylococcus aureus, salmonella typhi, Candida albicans* and *Escherichia coli* in clinical fields informed their choice for the study. These clinical isolates were collected from Federal Teaching Hospital (FETHA) II and transported using Nutrient broth to the Applied Microbiology Ultra-Modern Laboratory of Ebonyi State University, Abakaliki, Ebonyi State. 500 mg each of amoxicillin, ampiclox, ciprofloxacin capsule and 200 mg of ketoconazole tablets were purchased from winners' pharmaceutical shop at Presco Campus Ebonyi state university, Abakaliki.

Preparation of reagents

Drug samples

Approximately, 0.042 g of amoxicillin, 0.053 g of ketoconazole, 0.042 g of ciprofloxacin and 0.05 g of norfloxacin were measured from the pulverized samples and each dissolved in 100 mL methanol to give 1 mmole of the drug.

Nutrient agar (TM media)

28.00 g of nutrient agar in 1000 mL distilled water was gently heated to dissolve completely and was sterilized by using autoclave at 15 psi (121°C) for 15 minutes. The medium was dispensed as required.

Nutrient broth(TM media)

13.00 g of the sample in 1000 mL distilled water was gently heated to dissolve the medium completely and was sterilized by using autoclave at 15 psi (121°C) for 15 minutes and cooled at room temperature before use.

Mueller Hinton agar(TM media)

13.00 g in 1000 mL distilled water was gently heated to dissolve the medium completely and was sterilized by using autoclave at 15 psi (121°C) for 15 minutes and cooled to 45-50°C prior to dispense.

Metal solution

0.273 g of cobalt (II) chloride hexahydrate was dissolved in 1 dm^3 of ethanol to give 1.00 millimole of $CoCl_2.6H_2O$.

Preparation of cobalt (II)-drug complexes

20.00 mL of 1.00 mmole of the cobalt(II) solution and 40.00 mL of 1 mmole the ligands (amoxicillin, ketoconazole, norfloxacin and ciprofloxacin) were mixed and stirred for about 3-4 hours with a magnetic stirrer at room temperature

and allowed to evaporate slowly in an open beaker for one week. Crystals formed were washed repeatedly with ethanol to obtain clear crystals. The Co(II)-amoxicillin and Co(II)- norfloxacin complexes gave golden green crystals while Co(II)-ketoconazole and Co(II) - ciprofloxacin yielded light blue crystals.

Inoculation of the test organisms (Agar Well Diffusion Method)

0.1 M, 0.01 M and 0.001 M solution of the Co(II)- drug complexes were made by dissolving the crystals in acetone and the samples labeled A, B, C respectively indicating different microbial inhibitory concentrations. The control has concentration of 0.1 M and working under antiseptic condition, six petri dishes containing the gelled Muller-Hinton agar was labeled appropriately according to the number of the test tubes. Using a sterile swab stick, the test organisms were inoculated by streaking and labeled in accordance with the name of the streaked organisms. Using flamed cork-borer, four holes A, B, C and control was bored on the surface of each plate medium and labeled according to the labeled test tubes. Using a sterile syringe or pipette, 1 mL of the homogenized solution from the various test tubes were inoculated into each hole on the various labeled petri dishes and allowed to stand for one hour before incubating at 37 ^{0}C for 18-24 hours after which the results were observed. The samples A, B, C and the control drug solutions were added into bored holes and incubated for about 18-24 hours after which the zones of inhibition of each sample was measured.

Results and discussion

The UV-visible spectra of amoxicillin, ketoconazole and their cobalt(II)complexes are represented in Table 1. From Table 1, it could be observed that amoxicillin , ketoconazole, amoxicillin Co(II)complex and ketoconazole Co(II)complex showed absorptions at 259,276 and 307nm, 221,245,275 and 295nm, 376.5nm and 276nm and 296.5nm respectively. The UV –Visible Spectra of norfloxacin, ciprofloxacin and their Co (II) complexes are also presented in Table 1. It could be observed from Table 1 that norfloxacin, ciprofloxacin, norfloxacin Co (II) complexes and ciprofloxacin Co (II) complexes showed absorption at 283 nm and 255nm, 309nm, 283nm and 253nm, 323nm, 276nm, and 250nm and 301nm, 276nm and 254nm respectively. The absorption band at 259 and 276nm could be assigned to π-π transition of the benzene ring and imine chromophore (C=N)(Adekunle et al., 2006; Li et al., 2001 and Skyrianou et al., 2009). The band at 307nm was attributed to π-π* transition of non bonding electrons in azomethine of the ligand (intraligand charge transfer (CT)) (Skyrianou et al, 2010). In the amoxicillin complex, the band at 307nm in the spectrum of the ligand was red shifted to376.5nm on the complex in the form of ligand to metal charge transfer transition. For ketoconazole, the absorption band at 221,245 and 275nm was as a result of π-π* transition of the benzene ring and imine chromophore whereas the band at 295nm was due to π-π* transition (intraligand charge transition). In the ketoconazole Co(II) complex, the band at 295nm on the ligand suffered bathochromic shift /(red shift) to 296.5nm as a consequence of ligation of the metal to the ligand(ketoconazole)(Shaikh et al., 2007; Sheikhshoaie et al., 2012).

Table 1. Uv- Visible (absorption) spectra of amoxicillin, ketoconazole, ciprofloxacin and norfloxacin and their complexes.

Compounds	Λmax^1(nm)	Λmax^2(nm)	Λmax^3(nm)
Amoxicillin	307(s)	276(sh)	259(s)
Ketoconazole	295(sh)	275(s)	245,221(s)
Amoxicillin complex	376.5(br)	-	-
Ketoconazole complex	296.5(sh)	276(s)	-
Norfloxacin	283 (sh)	255 (s)	–
Ciprofloxacin	309 (s)	283 (sh)	253 (s)
Norfloxacin Complex	323 (s)	276 (sh)	250 (s)
Ciprofloxacin Complex	301 (sh)	276 (s)	254 (s)

sh =shoulder, S= sharp, br = broad

The absorption at 283nm and 253nm could be assigned to π-π* transitions. These transitions occur in case of unsaturated hydrocarbons, which contain carbon atom attached with oxygen atoms as in carboxylic and ketone groups (Efthimiadou et al., 2006). In the norfloxacin, the band at 283 nm in the spectrum of the ligand was red shifted to 323nm on the complex in the form of ligand to metal charge transfer transition.

In other to study the binding mode of both the ligands to the metal in the complexes, the IR spectra of the uncomplexed ligands were compared with the spectra of metal complexes. The IR spectra of norfloxacin and ciprofloxacin showed band in the region 3404 and 3442 cm^{-1} respectively assignable to the OH group. The absence of these bands in metal complexes reveals the deprotonation of the OH group and the involvement of the oxygen atom in complexation. The IR spectra of norfloxacin, ciprofloxacin, norfloxacin complex and ciprofloxacin complex showed band in the region 1999, 1937, 1968 and 1902 assignable to C=O. Also the IR spectra of norfloxacin, ciprofloxacin, norfloxacin complex and ciprofloxacin complex showed band in the region 1470, 1505 and 1566; 1400, 1381, 1439 and 1466; 1624, 1597, 1624 and 1628; 1254, 1258, 1258 and 1227 assignable to C=C, C-N, C=N and C-O

Table 2. In vitro antimicrobial evaluation of norfloxacin and ciprofloxacin and their Co(II) complexes

Compounds	Microorganism's Zone of Inhibition (mm)											
	E. coli			*Staph. aeurus*			*Salmonella*			*Vibro*		
Concentration	0.1	0.01	0.001	0.1	0.01	0.001	0.1	0.01	0.001	0.1	0.01	0.001
Norfloxacin Complex	24	23	20	08	08	08	25	21	20	24	24	22
Control	20			06			18			20		
Ciprofloxacin Complex	26	26	25	20	20	18	19	19	18	24	24	18
Control	28			19			15			20		

respectively. According to the IR spectral data, the norfloxacin and ciprofloxacin was coordinated to the metal ions as a bidentate ligand through one-carboxylato oxygen atoms and the oxygen atom of the pyridine carbonyl group (Turel, 2002; Ture et al., 2003).

Table 3. In vitro antimicrobial evaluation of the amoxicillin and ketoconazole and their Co(II) complexes

Compounds	*Microbial zones of inhibition*								
	E. coli			*Salmonella*			*Candida*		
Concentration	0.1	0.01	0.001	0.1	0.01	0.001	0.1	0.01	0.001
Amoxicillin	10	08	08	08	08	08	-	-	-
Complex									
Control	06			07			-		
Ketoconazole	-	-	-	-	-	-	25	24	21
Complex									
Control	-			-			20		

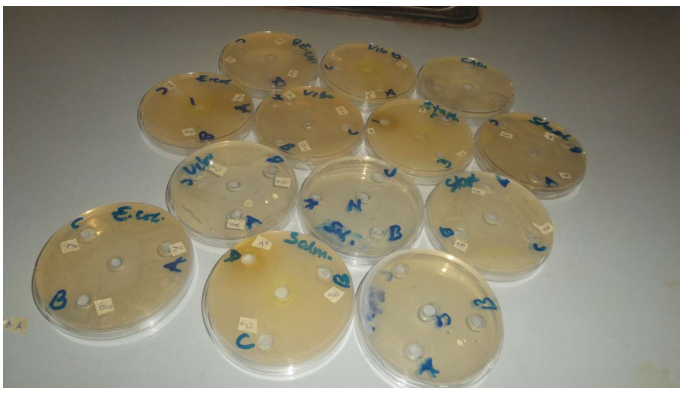

Figure 5. Zones of inhibition of drugs and their Co(II) complexes.

The IR spectra of amoxicillin, ketoconazole, ketoconazole Co(II) complexes and amoxicillin Co(II) complexes showed broad band at 3539,3504,3570 and 3477cm^{-1} respectively due to stretching vibration of co-ordinated water. The free OH(hydroxyl, phenolic)vibrations was observed at 3230,3137,3276 and 3269cm^{-1} for amoxicillin , ketoconazole, amoxicillin Co(II) and ketoconazole Co(II) respectively. The C=N stretching frequencies was observed at 1659,1655,1686 and 1659cm^{-1} respectively for the amoxicillin, ketoconazole, amoxicillin Co(II) and ketoconazole Co(II) respectively. There was a shift in the C=N band for amoxicillin from the 1659 to 1686cm^{-1} as a result of complexation. Similarly, C=N for ketoconazole was shifted from 1655 to 1659cm^{-1} as a result of complexation (Talan, 2001). There was appearance of new bands at 544 and 613cm^{-1} and 547cm^{-1} respectively for amoxicillin Co(II) complexes and ketoconazole complex which was absent in the ligands/drugs indicating the ligation/complexation of the metal to the ligands/ drugs in the form of Co-O bond respectively (Thomson, 2000; Turel et al, 2003).

The inhibition zone diameter (IZD) and minimum inhibitory concentration (MIC) of the ligand and complexes are given in the Tables 2 and 3. The antimicrobial activity was performed by measuring the diameter of the inhibition zone and the screening results are given in Tables 2 and 3. The results showed that the metal complexes are more effective as antibacterial agents as compared to the uncomplexed ligand (control) in all the concentrations. This increase in the activity is being considered due to increased bioavailability of the metal drug complexes and the aqueous solubility of norfloxacin and ciprofloxacin. Such increased activity of metal chelate can be explained on the basis of the overtone concept and chelation theory (Mohamed et al., 2006). According to the overtone concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only lipid-soluble materials regarded as liposolubility an important factor that controls the antimicrobial activity. On chelation the polarity of the metal ion will be reduced to a greater extent due to overlap of ligand orbital and partial sharing of the positive charge of

the metal ion with donor groups (Tumer et al., 1999). Amoxicillin complex showed improved antimicrobial activity against E. coli when compared to that of ligand as the ligand showed no activities against salmonella typhi when compared to that of complex of amoxicillin as shown in the MIC result. Co(II) complex of amoxicillin showed decrease in antimicrobial inhibition when compared to those of ketoconazole and the ligand. This could be as a result of loss of some necessary pharmacophoric moieties due to co-ordination with the metal ion.

Conclusion

In this work, in vitro antimicrobial studies carried out against clinically important micro-organisms E. coli, Staphylococcus aureus, Salmonella typhi and Vibro, the complexes were found to possess better activity against all the microorganisms tested than the uncomplexed ligands (norfloxacin, ciprofloxacin, ketoconazole and ciprofloxacin). It was concluded that the obtained complexes could be a better alternative in the treatment of diseases ailments associated with such microbes.

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